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(57) Abstract

The present invention provides method and means for synthesis of components comprising affinity groups, structural members and optionally functional members into assemblages having well controlled structure on the molecular and atomic scale. Methods are provided for the hierarchial construction of components and assemblages thereof which expand assemblage structure in a well controlled manner while retaining controlled addressability of affinity groups through differentiation of the specificity of affinity groups. Methods for the immobilization of such components or assemblages thereof to solid phases, particles, surfaces, actuators and sensors in well controlled manners are provided. Additional methods and means for effecting the controlled colocalization of objects comprising the components of the present invention are provided. The present invention has broad applicability to several technologically and industrially critical applications, as demonstrated by specific embodiments of the present invention useful in diverse applications.

ASSEMBLY OF COMPLEX MOLECULAR AND SUPRAMOLECULAR OBJECTS AND DEVICES AND USES THEREOF

TECHNICAL FIELD OF THE INVENTION:

The present invention concerns the construction of molecular and supramolecular objects and complexes with well controlled structure, devices therefrom, and uses thereof.

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Selected Modes for carrying out the invention:

XV. PREFERRED EMBODIMENT: MATRICES AND SCAFFOLDS FOR MATERIALS FORMATION AND NANOSCALE PRECISION STRUCTURING

Notes

* * *

BACKGROUND ART:

The vast array of complex molecules which make up living organisms, upon whose dynamic functioning each organism depends, provides examples of molecules with well defined structures that perform manipulations and transformations of other molecules, often in exquisite coordination with yet other molecules, in a manner that is highly responsive to the needs of the total system and requirements imposed by the environment. Some such molecules and molecular complexes may be described as naturally occurring molecular machines, representing an existence proof for the category molecular machines. Among these are examples of molecular complexes with well defined heterogeneous, or non-regular, structures not readily attainable by conventional chemical synthetic techniques heretofore known within the appropriate arts. The construction of analogous synthetic heterogeneous extended molecular structures of arbitrary design is a prerequisite to the fabrication of complicated molecular devices and machines with diverse technological uses.

There has been a vast and fruitful effort to modify and harness such biological molecules for artificial utility in numerous areas including industrial chemical production, agriculture, scientific research and medicine; this area is generally referred to as biotechnology. There are also significant efforts underway towards employing naturally occurring and also modified biological molecules, namely bacteriorhodopsins, in information storage and computation, though these areas are as yet in early stages.[1]

Other purely synthetic classes of molecules, so-called super-molecules or supra-molecular complexes, have been produced with the goal of use in pharmacology, medicine and other technological uses, as well as in scientific research.[2,3,4] Of

particular note here are artificial receptor molecules, artificial enzymes (molecular catalysts) and stereoisomer purifying (resolving) agents.[5]

There are further various efforts to produce biologically derived or non-biological molecular compounds capable of functioning as switches or rectifying devices, towards the goal of a molecular electronics that relies on individual molecules rather than large ensembles of molecules to perform switching and amplification functions with utility similar to that of microelectronics, in both digital and analog applications, but deriving additional advantage from the increased device density, device speed and inherently lower power consumption per operation that inheres in devices of nanometer scale.[6,7,8] Such technologies will require methods for suitably arranging such molecules within a controlled structure, for suitably interconnecting such molecules, and for addressing individual molecules or devices.

Similar advantages, including towards such applications as data storage, information processing and machine control may be gained from devices capable of transforming their state in, for example, deterministic ways that do not necessarily involve the motions of electrons or electron deficient "holes", but represent data, analog or digital, by some physical or chemical state or a molecular or supramolecular structure or complex or assemblage.

A significant portion of biotechnological art concerns the production and modification of specific proteins. One significant limitation to the application of biotechnology to non-biological uses is that ex vivo many proteins are of limited stability and durability. Further, despite promising recent steps in the field of protein structure prediction[9,10,11], the goal of which is to predict the three dimensional configurations assumed by a protein molecule from the linear sequence of amino acids of which it is composed, and in the related field of protein design, most protein molecules of interest are of a size that confronts such efforts with a vast and hence highly challenging complexity. In the field of rational design, this complexity has been approached by methods such as those taught by K.D. Hardman in U.S. Patent Number

4,939,666, requiring substantial effort for each molecular configuration to be evaluated, or by other such computation intensive methods.

Because of the relationship between the structure and function of a protein, lack of structural information about many natural and modified protein molecules of interest has impeded efforts to design proteins with novel functions. For similar reasons, despite the internal structural heterogeneity of polypeptide and protein molecules, there has been little significant progress in the construction of extended heterogeneous but well controlled and regionally addressable structures from proteinaceous building blocks, and no general capability of this sort based on proteins subjected to rational design has yet been demonstrated.

One synthetic method which has addressed the chemical synthesis of biological macromolecules is found in the teachings of K. L. Beattie and B. White in International Publication Number WO 90/00626 (PCT/US89/02915). Here, methods permitting the segmental construction and remodeling of biopolymers are described. Such methods are sometimes termed convergent syntheses by those skilled in the chemical arts. These methods compare favorably in some regards to the construction of desired biopolymers by the serial, stepwise addition of monomers, but are applied by these inventors only to some biopolymers and also do not offer very much help in constructing the kinds of predetermined heterogeneous structures of technical interest which are of present concern.

I. PRIOR ART CONSTRUCTION OF MOLECULAR GEOMETRICAL OBJECTS:

One biotechnological approach to producing structures of non-biological technological interest, found in the teachings of N.C. Seeman in U.S. Pat. No. 5,278,051, distinguishes itself with a rudimentary ability to produce heterogeneous structures. Here polynucleotides are used in the construction of extended molecular structures such that predetermined structural heterogeneity may be realized. This approach, however, carries with it limitations inherent in the properties of polynucleotides, limitations on the compositions which may be

produced by the method, and limitations stemming from the exact methods of construction. The method of these teachings is limited to producing objects wherein the covalent connections between building blocks occur through the phosphodiester bonds formed by ligating together both strands of the sticky ends of polynucleotides which were in turn formed either synthetically or by the cutting action of a DNA restriction enzyme at its target sequence in double-helical DNA. While this disclosure defines polynucleotides in a very broad manner comprehending molecules substantially different (including unusual compositions of the polymer backbone) from those which might be found in nature, the objects disclosed and any objects constructed by that procedure from polynucleotides which will serve as substrates for the restriction enzymes (termed growth enzymes in that disclosure) necessarily employed in this method have numerous conformational degrees of freedom. This precludes the construction of structures which have a high degree of rigidity. Rigidity may be desirable with respect to the physical properties of the objects or structures being produced, for instance their response to applied forces. The ability to produce and modify structures with controlled rigidity is also advantageous with respect to the process of designing specific synthetic routes to particular desired structures and objects, for instance as a constraint on the interaction of chemical functional groups within the structure under construction. Because this method takes polynucleotides as an obligatory articulation material covalently joining building blocks, as well as a preferred structural material, construction materials will suffer from the expense of synthesis and synthetic reagent instability associated with polynucleotides. Although these are not prohibitive, they pose important limitations on the fabrication of complex constructs. Additionally, because this approach necessarily relies on the use of restriction enzymes and polynucleotide modifying enzymes, the solvents in which construction may be conducted are limited to those in which the enzyme will remain active and retain its specificity. This limitation becomes crucial when a desired construct is to include molecular components which are insoluble in solvents required by restriction and ligation

enzymes. In such circumstances, more powerful methods than those taught in this disclosure are requir d. It must also be noted that dependence on restriction enzymes generally limits addressability to the sequences recognized by available restriction enzymes. Exclusive reliance on "sticky-end" restriction overhang limits the targeting and subsequent linkages formed between precursor molecules to the limited resolution of selectivity or discrimination that is possible for targets of less than six, and often less than four base pairs. This poses a limit on the number of distinct types of targeted linkages can reliably be formed simultaneously in a practical manner. Further, the method taught therein relied on denaturation and treatment with exonuclease to eliminate "failure products," spare reaction products which have undergone the desired addition from degradation, and recycle unreacted reactant molecules. Though useful, these steps do not provide a method of checking or selecting products according to more than one property, that of strand or loop closure. It would be desirable for a construction method to enable one to check for the presence and/or relative position of several component structures within a product structure, and to purify product molecules accordingly, especially if recycling of unused reactants could concurrently be effected.

II. PRIOR ART POLYMER SYNTHESIS METHODS:

A. DENDRIMERIC POLYMERS:

A class of polymers having many branches from a central or seed monomer have been developed in the last two decades. These compounds are polymerized according to a variety of methods but tend to be formed by a process of polymerization with concurrent branching such that as the radial distance from the central or seed monomer increases, the density of monomers on the surface of an imaginary sphere centered on the seed monomer increases, reaching a surface density which is limits the addition of further monomers. The polymerization thus yields substantially monodisperse particles. Other methods have also been employed in the production of dendrimers and related molecules. This class of polymers has been discussed

extensively in the literature. For a review[12] of this area se Tomalia, 1994.

B. PRECISE LENGTH POLYMERS:

Only recently have methods been disclosed for the synthesis of monodisperse polymers of exact molecular length (i.e. the production of a population of polymer molecules all of which are of an identical number of monomers in length) and precise sequence[13], and precise branching.[14] These authors teach that by protecting the ends of monomers with chemical functions which are each removed by different treatment, and by removing one of the two protecting function from each two aliquots of the reactant monomers to be reacted together, an exact doubling in length is obtained per reaction cycle. The product of such a reaction will have the different protecting groups (the ones not removed from each of the two aliquots of monomers before their reaction together) on each end. By applying this method recursively (taking the product from one such reaction and based upon its length, using it as a macromonomer with the same reactive chemistry as the parent monomer), reaction products with homogeneous and numerically precise length may be obtained. With this method, increase in product size proceeds at a maximum rate of 2^n monomers per n reaction cycles. With these techniques, purification of the larger product is facilitated by the large proportionate size difference between reactants and product (though the authors do not note this advantage.) These methods, while an important advance over the conventional methods of polymer chemistry which yield mixtures of polymerization reaction products which have a statistical distribution of lengths, still suffer from important drawbacks. The precision gained by this method is gained by choosing monomers with different protecting groups at each of two distinct ends which may be selectively removed to prepare the monomer for reaction with the complementary activated functional group of the conversely treated monomer. The polymerizations to which this method applies are therefore required to be unidirectional, with component molecules joined together in one invariant polarity or orientation. Because control over length depends on control over deprotection of the

reactive functionalities of monomers, this control is limited to the conditions which may varied simultaneously and the responsiveness or specificity of the protecting groups to these conditions. This is to say that where there are more than just a few different reactive functionalities to be differingly protected, painstakingly designed and assiduously executed protection schemes will still only permit a limited degree of addressable control of the reactivity of particular reactive groups within a molecular structure or complex. By the term "addressability", the precise selection of one of several potentially reactive sites on a molecular construct for deprotection and/or reaction with some other reactant is meant. A greater degree of addressability would facilitate the construction of more complex objects and structures, increase the efficiency with which the desired product may be produced from reactants, and could reduce the number of steps required to obtain an object with a given desired structure. Further, these authors have taught[15] that their methods are mutually exclusive with the solid-phase synthesis methods pioneered by R. B. Merrifield[16,17] and characterize the methods they disclose as generally much more efficient. Thus, the advantages which may be gained with solid phase synthesis techniques, including the facilitation of recycling and washing away unused reactants, and rapid recovery and transfer of the molecular object being constructed between different solvents and reactive media without undue dilution or unnecessary manipulations, are not availed within their methods.

III. SOLUBLE RIGID POLYMERS:

Most polymers are composed of monomers bonded together covalently through a single covalent bond (not collinear with the next) which admits some rotation of adjacent monomeric units relative to each other, with some angular movement of the polymer backbone. Thus most polymers are rather flexible at the molecular level, and may assume a very large number of conformations. There are a large number of polymers which by contrast have monomeric units which themselves have no internal rotational degrees of freedom, and either have more than one bond between adjacent monomeric units or are linked together by

bonds that lie along the same line. Polymers with these characteristics are rigid at the molecular level, and materials composed of polymers of this type tend to have high tensile strength. By virtue of having few internal degrees of freedom, at the molecular level rigid polymers have properties similar to those of solids, and individual molecules readily associate and thus precipitate as insolubles. With very few exceptions, rigid polymers are insoluble in solvents usually used for polymerization and processing. Recently, a few distinct methods have been developed to solubilize rigid polymers. These include the method disclosed by S.A. Jenekhe and J.R. Peterson in U.S. Patent Number 5,114,610, which describes a class of solutions which are particularly favorable to the solubility of such polymers, and the experimental method[18] described by M. Rehahn et al. for derivatizing rigid polymers or their precursors to render larger polymers soluble in various solvents.

A number of the polymers studied by the latter method are of keen interest as conducting polymers for molecule or polymer based electronic technologies. Prominent examples include polyparaphenylene and derivatives,[19,20] and linear alkali fulleride[21].

IV. PRIOR ART TOPOLOGICAL COMPOUNDS INCLUDING ROTAXANES, CONCATENANES, SHUTTLES AND SELF-ASSEMBLED TUBULES:

A class of substantially tubular semi-rigid polymers is comprised of macrocyclic compounds polymerized while encirling a linear polymer (complexes referred to in the chemical arts as rotaxanes) with more than one bond between adjacent macrocycles. These have been termed nanotubes.

Rotaxanes may be formed either by the random occurrence of the threading of a macrocycle by a linear polymer (statistical rotaxanes)[22] or by energetically favored interactions involving, for example, van der Waals and London energies (self-assembled rotaxanes). Some important examples of self-assembled rotaxanes are those of A.C. Benniston and A. Harriman[23], the many rotaxanes, concatenanes, pseudorotaxanes, shuttles and so-called molecular machines of J.F. Stoddart et al,[24,25], the

cyclodextrin rotaxanes and multistrand inclusion complexes studied by Akira Harada[26,27], and other cyclodextrin rotaxanes studied by G. Wenz and B. Keller[28]. Once threaded on a suitable host or template polymer, cyclodextrin molecules may be polymeriz d by reaction with epichlorohydrin and hydroxide[29] to form multiple ether linkages between adjacent macromonomers. The template polymer may later be extracted from this polymer, yielding a tubular compound. Such a tubular compound may in turn act as a host, accomodating guest molecules within the tubular interior. Guests in such inclusion complexes my thus be transported through liquid phases in which they would otherwise be insoluble. The tubular structure of such compounds has suggested their use as molecular devices[30,31] but no concrete proposals have addressed their assembly into larger structures or devices; in particular, there has been no clear proposal as to how such supermolecules may be "wired" to other structures.

Another example of so-called nanotubes has been synthesized by R. Ghadiri et al.,.[32]

V. RELATED ART MICROWIRES AND NANOWIRES:

Microfabrication techniques may be employed to etch wires of micron and submicron width from a large number of metals and other conductive materials. These techniques may also be employed to coat such wires with compounds suitable for reaction with other chemical compounds in solution, where said coating may occur in a spatially controlled manner. Finally, lift-off techniques, usually involving the dissolution of an underlying sacrificial material which served as the substrate for the microfabrication of said wires can yield wires in suspension in a solution. Such methods are generally well-known to those skilled in the arts of microfabrication and microlithography.

Linear and branched structures of similar scale have also been produced from conducting polymers by various means. Examples include the alternate electropolymerization of thiophene between particular electrodes selected out of a larger array according to voltages applied, to form specified connections.[33]

Lipid tubules have been used to templat the formation of metallic wires of somewhat controlled dimensions by chelation of metal ions and by electroless metallization. Similar techniques have been applied to proteinaceous fibrils.[34]

Self assembled linear structures comprising liquid crystalline molecules with pthalocyanine moieties have been prepared to micrometer length by C.F. van Nostrum et al.,.[35] These authors argued that such structures are likely to be capable of transporting electrons and ions.

Further, natural carbon fibers have been studied with a view towards use as nanoscale wires[36] It is conceivable that established methods could successfully plate such fibrils with metals at spatially controlled locations and self assembling monolayers, for example of organothiols, formed on these metals could mediate chemical linkages.

VI. PRIOR ART SPECIFIC BINDING AND BINDING AFFINITIES:

Within the arts of biochemistry and of synthetic chemistry, there are numerous examples of molecules with binding affinity for each other, with varying degrees of specificity. The strength of association between two or more molecules, as well as the specificity of the affinity interaction, is often subject to variation with multiple physical and chemical parameters. Affinity of this kind is the result of molecular recognition, which is the basis of molecular self-assembly phenomena[37] both in nature and in synthetic systems.

Molecules displaying these kinds of affinities include sufficiently complementary polynucleotides, interacting proteins, receptors and their respective ligands, enzymes and one or more of their respective substrates, enzymes and the appropriate transition state analog compound, antibodies including monoclonal antibodies and their respective epitopes or haptens, chelators and the ions they coordinate, carcerands and the molecules they bind, and polymers self-assembled around some molecule (referred to as mol cular imprinting)[38,39,40,41] for which specificity is desired, among others. In many cases, variations in biding specificity and strength are relatively well studied, as for antibodies and antigens, complementary polynucleotide sequences, and DNA binding proteins and their

target sequences (e.g. relaxation of the target sequence specificity of a class II DNA restriction enzyme such as Eco RI in the presence of glycerol at concentrations exceeding 5% v/v in otherwise optimal buffer.) There are also well established techniques for determining the cross-reactivity of a given first molecule with affinity for some second molecule with yet other molecules of varying similarity. It is often important to characterize such cross-specificities to determine the applicability or usefulness of such a first molecule for a given task. Numerous methods are known to those skilled in the relevant arts whereby one may determine the binding specificity, binding strength and cross-reactivity of given affinity molecules for given target molecules or materials. In some instances, it is desired that specificity keenly discriminate a unique target from any similar molecules or molecular regions (e.g. as with DNA restriction enzymes), whereas in other instances it is desired that a given specificity cross-react with all molecules that bear some requisite degree of similarity (e.g. antibodies or oligonucleotides used to recognize molecules from one species of organism based on recognition of analogous or homologous molecules from a different species).

A special case which routinely occurs in recombinant DNA technology is the specific binding of enzymes to double helical DNA, cleavage of DNA at precise sequences by a subclass of such enzymes known as restriction enzymes, and binding of DNA molecules thus cleaved and linearized to each other according to the terminally exposed single stranded sequence thus produced and the Watson-Crick base pairing complementarity rules governing the association of such fragments under appropriate conditions, usually followed by treatment with one of a class of enzymes known as ligases which form covalent chemical bonds which join the termini of DNA molecules thus associated at the appropriate chemical groups. This practice demonstrates at least two distinct instances of affinity directed association (that of the enzyme with a substrate, and that of complementary cohesive DNA termini with each other) and the formation of one type of covalent bond directed by an enzyme. These methods have been applied routinely and

successfully for many years, but in standard practice remain particular to manipulations of linear or circular DNA molecules.

VII. PRIOR ART METHODS FOR IN VITRO SELECTION OF BINDING AFFINITIES FROM LIBRARIES:

In recent years, various methods for obtaining oligomeric polymers with desired binding specificities and have been developed. These methods depend on the production of a library containing a large number of molecules with random sequences of monomers. These libraries are then subjected to some form of retentive selection based upon specific binding properties of individual molecules. These molecules are usually biopolymers, and most frequently polypeptides or polynucleotides. This is both because nearly all applications of such molecules have been in fields related to biology such as the development of pharmacological agents and the development of medical diagnostic reagents. The choice of biopolymers as the objects of such evolutionary techniques is also motivated by the ability to have the sequence of the potential binding affinity molecules sought specified in genetic material that may be amplified in vitro and in vivo. Such systems also carry the advantages of permitting the sequence of the obtained binding affinities by such established methods as DNA sequencing, and of facilitating mutational approaches to the refinement of binding affinity or specificity. In other words, a system wherein the sequence (and hence structure and properties) of a binding affinity group is in some way determined by genetic material is susceptible to recombinant DNA techniques in addition to the selective (e.g. "panning")[42] or evolutionary[43] techniques permits a combination of both rational and so-called irrational (i.e. based on random and unspecified molecular structure rather than on detailed structural knowledge) design. These methods for obtaining molecular binding affinity groups are particularly attractive because the ultimately desired binding specificity is obtained operationally through the selection steps. These methods may be applied to different kinds of specific affinity interactions, including immunoglobulin-epitope/hapten, enzyme-substrate, enzyme-

transition state analog, ribozym -substrate[44], polynucleotide-small ligand[45,46], and DNA-protein[47]. As will become clear in the disclosure of the present invention, these advantages may be more fully exploited by the addition of novel steps to the selection procedures and the design of additional levels of variation and versatility into the structure of the genetic material that specifies the population of molecules subjected to these evolutionary methods.

VIII. RELATED ART MOLECULAR STRUCTURES INCORPORATING MULTIPLE NATURAL AFFINITY GROUPS:

In U.S. Patents Numbers 5,168,057 and 5,196,351, C.S. Oh et al. disclose bidentate and trifunctional conjugates, respectively. These conjugate molecules are structures incorporating naturally occurring small molecule affinity groups or small molecule fragments obtained from larger molecules for either the association or the prevention of association and thereby colocalization or prevention of colocalization, according to the embodiment selected, of distinct naturally occurring affinity groups, for analytical purposes, such as assays for the detection of the presence or absence of some analyte and/or determination of the concentration of some analyte. These conjugates are produced, according to the specificity desired for the desired assay use, with close attention to the affinity properties of the Such structures are used by these inventors in a manner that forms precipitated products which do not display any long range controlled ordering. These products are generally amorphous precipitates. Thus it not possible to use the methods of these inventions nor, generally speaking, the articles disclosed in these inventions, to produce extended molecular objects or assemblages with controlled heterogeneous (or well controlled homogeneous) structures. The complexes formed in these inventions are of an amorphous character, which suffices for the macroscopic diagnostic assays (e.g. nephelometric or turbidimetric assays) with which these inventors are concerned. It is essential to note that the primary concern of this invention is a limited number of affinity interactions which yield chain reactions (such as precipitations) as opposed to

the well controlled association and linkage of structural members and/or functional members. No application of the methods taught nor th articl s disclosed therein to the construction of any form of molecular or supramolecular device contemplated by these inventors.

IX. PRIOR ART POLYMERS INCORPORATING AFFINITY GROUPS:

L.J. Fetters et al. disclose polymers with associative end groups attached to central polymer chains in U.S. Patent Number 5,238,643, which they refer to as telechelic polymers. In particular, these molecules are used to produce polymer materials with a low degree of entanglement of the constituent polymer molecules. This invention aims at the production of high elongation elastiomers and highly oriented films and fibers, i.e. polymeric materials wherein the properties of interest occur at the macroscopic level.

The telechelic only homophilic affinity interactions are employed (i.e. affinity without binding selectivity, where the association of the end groups is based on a preference of like for like moieties), and the associating functions or blocks used are non-specific physical affinities that respond, for example, to solvent composition. In this invention, solvent composition (e.g. polarity, pH) or temperature is varied at appropriate times leading to precipitation of one of the two types of segments of the block copolymers of the invention. End-wise physical association of the macromonomers of that invention occurs. The types of affinity groups used in this invention are thus non-specific, which is to say that one such affinity group is not capable of selectivity in its association with two different affinity groups of the same type but different sequence or configuration. Further, there is no disclosure of the use of combinations of distinct macromonomers, or of precision control of the structure (e.g. length in monomers) of the production of distinct assemblages from a well controlled number of such macromonomers. In sum, the molecules of this invention are incapable of addressability or programmability at the level of molecular recognition or self-assembly. These methods are employed with the sole aim of reducing the degree of entanglement, and increasing the degree

of anisotropy by stretching, of polymer solids) Thus this invention is concerned with th production of polymeric materials with particular macroscopic properties, rather than extended molecular structures with controlled heterogeneity or devices of such type.

The telechelic affinity functions of this invention are linked exclusively at the termini of linear block copolymer molecules. Thus, such molecules form only larger linear chains and do not provide any basis for the formation of even slightly complex structures.

Selected Modes for carrying out the invention:

RELATED ART METHODS FOR PURIFICATION:

A. PURIFICATION BY AFFINITY ASSOCIATION:

In the arts of molecular biology and biochemistry, methods are also known whereby molecules are separated or purified based upon such an affinity interaction as described above. These methods are generally termed affinity purification (AP) techniques, and include affinity chromatography (AC) techniques. Affinity purification techniques have by now become an important tool within the biochemical, molecular biological and biotechnological arts. Common examples include immunoaffinity purification, oligo-dT chromatography, oligonucleotide purification, and various affinity conjugation techniques. These methods depend on affinity interactions either somewhat retarding the transport of molecules through a matrix or complete retention of affinity bound molecules in that matrix under appropriate conditions. One example of such purification is disclosed by J. Van Alstine in U.S. Patent Number 5,108,568. In biotechnology and biological research, AP of peptides and proteins are routinely conducted with antibodies bound to a matrix or solid support, and AP of nucleic acids are routinely conducted with oligonucleotides or polynucleotides bound to a matrix or solid support.

Similarly, AP may be conducted with the ligand of a receptor which is to be purified; AP of an enzyme may be conducted with an inhibitor of that enzyme or by a chemical analog of the transition state of the reaction catalyzed by said enzyme. AP and AC of an enzyme may also be performed with the relevant

substrat under non-catalytic conditions. An example of such methods, with said substrate bound to a sepharose matrix, hav been described by H. Tanaka et al.[48]

An important type of AC relies on the conjugation of short peptides or ensembles of short peptides to chromatographic media is so-called paralog chromatography.[49]

B. PURIFICATION BY FLOW FRACTIONATION:

Methods have been developed for the labeling of particles including living cells with visual labels such as dye molecules including fluorescent dyes. These methods include the coupling of monoclonal or polyclonal immunoglobulins to dye molecules or dye labeled microscopic beads, with binding of said immunoglobulins detected by appropriate optical means and detection used to control sorting means. A prominent example of such methods is Fluorescence Automated Cell Sorting (FACS) whereby cells are labeled with such a dye labeled immunoglobulin, and a liquid suspension of said cells is separated into droplets which are coated with a layer of charged oil. Said droplets fall, pass through a detector and then between the plates of a capacitor which bear a charge which is adjusted in proportion to the intensity of photons of appropriate wavelength detected by said detector. The path of the falling said droplet is deflected according to the interaction of said charged oil with said plates of a capacitor, and the location within a collection apparatus at which said droplet lands is thus indicative of the degree to which said cell was labeled. Thus, such methods may be used both analytically or for purification. Samples need not be restricted to living cells.

Another set of methods, termed field-flow fractionation (FFF) may separate particles according to mass, size, density, charge, diffusivity and/or thickness of adsorbates, among other properties. These methods have been reviewed by J.C. Giddings.[50]

XI. PRIOR ART IMMOBILIZATION OF MOLECULES ON SURFACES:

Various techniques have been developed to reversibly or irreversibly attach molecules to solid surfaces with varying degrees of sp cificity. The literature discussing scanning probe microscopy is an abundant source of information on such methods. A convenient method relies on the formation of self-assembling monolayers (SAMs), particularly on metal surfaces. A well studied example of such associations is the formation of SAMs of alkanethiols or their derivatives on gold[51]. These compounds will generally also form SAMs on silver, mercury, zinc and nickel surfaces. These SAMs can be quite stable, for instance withstanding the several GPa of pressure usually applied by the tip of an atomic force microscope. Similar SAMs have been used with a gold surface as the initial layer of ~ultilayer ~helated metal structures[52,53] which may exhibit ;emiconductivity or conductivity.

Various methods have been developed, again in the field of scanning probe microscopy, to immobilize single molecules, particularly biological molecules, to gold surfaces and to mica surfaces with good reproducibility. In these instances the lateral stabilization of each adsorbed molecule inherent in a SAM structure is absent. A thiol-derived double helical DNA molecule may be bound to gold[54], suggesting a durable and well localized chemisorptive association occurs. Nucleic acids and enzymes associate with mica either directly or via divalent cations through ionic associations, which are also quite stable to the high local pressures exerted by an AFM tip.

Other examples include: the immobilization of enzymes to metal surfaces (especially enzyme-electrodes[55]); and the formation of thin (e.g. Langmuir-Blodget)[56] or thick films on solid surfaces including electrodes, which may in particular be molecularly imprinted, self assembled polymer matrices which will subsequently display some degree of selective binding affinity for molecules identical, or in other cases similar to, the molecule with which said polymer matrices were imprinted[57] during polymerization. Further, there is growing interest in the immobilization of polynucleotides to portions of the surfaces of microfabricated microelectronic devices such as charge coupled photodiode arrays for screening and diagnostic purposes in the fields of genomic biology.[58,59] Methods have

been developed to utilize photodeprotection chemistries and surface immobilization to effect lithographically controlled st p-wise polymerization of affinity molecules onto surfaces, with spatial features comparable to those of microfabrication. Depending on the molecules used and the devices produced, molecules may be immobilized with varying degrees of control over the type and configuration of binding both as these regard the linkages from the molecule and as regards the localization of specific molecules to specific regions of a device (as in the case of polynucleotides immobilized on integrated circuits.) It is possible, for instance, to genetically modify the carboxy-terminus of a cloned immunoglobulin molecule such that the purified molecule thus produced will bind a divalent metal such as nickel, forming a complex which will in turn adsorb to a charged solid surface (e.g. mica) via the divalent metal (i.e. via a specific portion of the molecular complex).[60] Subsequently, said immunoglobulin molecule thus immobilized on said solid surface may be used to bind and antigen or hapten or complex of molecules, thus immobilizing other molecules in a selective manner. Said other molecules may, for example, be enzymes or biological receptors. Thus, molecules, macromolecules and complexes of molecules which are capable of various affinity interactions have been bound to surfaces with varying degrees of (and means of) regiospecificity and varying means of chemical or physical attachment, in the fabrication of devices with diverse uses.

There have been some successful efforts to derivatize the surface of a scanning probe microscope probe (or tip) with molecules. One set of methods coats the tip surface with gold and then incubates with organic thiol compound to coat the tip to form some functionalized surface.[61,62,63]

Another set of experiments has relied on the strong non-specific association of bovine serum albumin (derivatized with biotin) and the silicon nitride commonly used to produce such microfabricated tips.[64,65]

A third approach, which has been disclosed by R. Kopelman et al. in U.S. Patent Numbers 5,148,307 and 5,264,698, describes the inclusion of a complex of dye molecules near the mouth of a fine glass capillary.

All of these examples concerning tip functionalization, while demonstrating distinct and durable immobilization methods, are in one sense or another amorphous: they do not yield a precise distribution of molecules (e.g. specific to the tip apex in the former two cases), nor precise molecular conformational structure or (in the latter case) molecular-complex geometry.

XII. RELATED ART POLYMERIC ELECTRONIC COMPONENTS, DEVICES AND CIRCUITS:

In recent years, integrated electronic components and devices, comprised of polymeric compounds rather than exclusively of solid state semiconductors, and corresponding process and fabrication technologies, have been described. These devices have been fabricated by printing methods and by lithographic or microlithographic methods on either solid or flexible substrates, but like conventional solid state integrated electronic devices, have been substantially confined to two-dimensional integration. Examples include those disclosed by M.S. Wrighton et al., in U.S. Patent Number 5,034,192, by M.S. Wrighton et al., in U.S. Patent Number 4,721,601, by F. Garnier et al.,[66] and by Y. Yang and J. Heeger.[67]

XIII. PRIOR ART ENERGY STORING AND TRANSDUCING ELASTIOMERS:

Polymeric molecules capable of storing and also of transducing (converting) various forms of energy have been investigated and demonstrated by Dan W. Urry.[68,69] These molecules have been termed elastiomers by that investigator, and have been shown to undergo first order thermal transitions as a function of changes in various physical conditions or physical stimuli. These compounds have therefore seen application in so-called smart materials. This class of molecule, however, has been utilized only in macroscopic materials and objects; the molecular mechanical properties of these compounds have been availed only for devices an articles many orders of magnitude larger than the molecules themselves.

Related phenomena have recently b en described in synthetic graft copolymers.[70]

OBJECT OF THE INVENTION:

A fundamental problem beyond devising suitable basic components (e.g. a molecule capable of performing switching functions) is the need for convenient methods of positioning these and other components in a predetermined spatial configuration with a predetermined connectivity.

Advantages in device density over existing miniaturization technologies, which at present are only capable of producing planar integrated devices in a commercially viable manner, would be gained by any miniaturization technology capable of practically exploiting integration of devices in three dimensions rather than two. Device density improvements would scale as the third power of the reciprocal of smallest feature size rather than the square of the reciprocal of the smallest feature size of a given miniaturization technology. At present, there is no well established miniaturization technology that can do in three dimensions what is routinely practiced in the production of two dimensional devices. One of the obstacles to the practicality of three-dimensional integration has been that the number of fabrication steps necessary to produce a three-dimensional integrated device with n layers generally increase by a factor of n compared to a one layer integrated device. Because fabrication costs are related directly to the number of devices produced in a given number of fabrication steps, there has not been a cost advantage which corresponds to the density advantage of three-dimensional integration. This is to say that microlithography and microfabrication methods are not amenable to layer- or plane-wise parallelism in the production of three-dimensional integrated devices. The number of fabrication steps scales linearly with the number of component layers incorporated into a three dimensional device. It would therefore be particularly advantageous to produce three-dimensional integrated devices with only a few additional steps beyond those required to fabricate two-dimensional integrated devices.

These and other technologies, which have in common a keen interest in highly precise or well controlled structured heterogeneity at the atomic, molecular or nanometer scale, have been grouped together in the category of nanotechnology. Technology at the scale of atoms and molecules was first proposed by Richard Feynman[71] and later elaborated and propounded by K. Eric Drexler[72,73,74] Nanotechnology is therefore a trans-disciplinary field involving elements from such diverse existing technologies as biotechnology and molecular biology, microfabrication including so-called nanofabrication which produces solid-state structures in the sub-micron regime[75], synthetic chemistry, supramolecular chemistry, polymer chemistry, biochemistry, chemical physics, materials science, scanning probe microscopy, computer and information science, molecular modeling, optics, and mechanical engineering as well as others. Applications and uses of such a technology are as diverse as those of all the fields which enable it. Further applications and uses extend to such uses as described by Drexler in the realm of general purpose, high flexibility, dynamically responsive industrial productive capital equipment.

One fundamental challenge posed in development towards such technologies, as well as technologies that manipulate and transform molecules and larger structures in a manner analogous to but possibly more flexibly than biological molecules, is the need for chemical methods capable of producing complex, extended, molecular and supramolecular structures with well controlled structural heterogeneity. Here heterogeneity shall refer to the same kind of structural differences between the individual parts of most microfabricated or macroscopic machines or devices, as opposed to the molecular level homogeneity or amorphousity which characterizes many solid materials. Ideally such methods would enable nanometer scale precision in the structural specification and molecular or atomic composition of any arbitrary article of manufacture or production, and highly refined control over the specificity with which such articles may connect or communicate with other structures or physical phenomena. Such methods would further make possible a new domain for the research of molecules and

molecular systems, as is suggest d by the increasing application of scanning probe microscopy to problems in scientific research. Onc such methods are understood and established, one may readily envision a hybrid technology for the nanometer scale that draws on the molecular construction methods of the present invention, the already well established technologies of microlithography and microfabrication, and positional manipulators like scanning probe microscopes or those described by Drexler (1991, 1992).. Methods that facilitate rapid production of, for instance, mole quantities of devices or products, especially those which may function as machines for the manipulation and modification of other molecules, and which have any self-replication capability[76,77,78] would represent substantial advances over existing technologies. Thus, technologies facilitating the realization of agile manufacturing concepts across a broad range of industrial activities, and associated manipulation technologies for research and development could improve a large portion of scientific, technological and industrial activity.

Related to this challenge of heterogeneous structural control is that of interfacing such nanometer scale systems with the macroscopic world. Many applications envisioned for molecular devices entail control and information transfer from the macroscopic to the molecular level. In recent years there has been increasing interest in research and experimental methods for the manipulation and observation of single molecules.[79,80,81,82] It would, for example, be highly desirable to place a molecule with a well known spatial and electronic structure precisely at the apex of a scanning probe microscope tip: this would provide a facile method for the preparation of substantially identical tips of sub-nanometer sharpness and high, well controlled aspect ratio. Conventional microfabricated or electron-beam d posited tips generally have apical radii of curvature of greater than 10nm, and frequently are sufficiently rough to display noticeable "multiple-tip" artifacts in images. Because scanning probe microscopy inevitably images tip-sample interaction, variation in images of identical samples can be no less than variation in tip

quality. Further, while some solid surfaces readily yield atomic resolution, any sample that is not atomically flat will typically interact with more than one atom of a tip, substantially degrading image resolution to approximately the radius of curvature of the tip, even in scanning tunneling microscopy. Thus molecules of precise structure and size positioned exactly at a tip apex stand to greatly improve the resolution and image quality that may be routinely achieved.

It should be noted methods accomplishing this goal could be generalized in various ways to the formation of precisely localized attachments of molecules or molecular assemblages to solid surfaces via transfer of molecules (or portions of molecular complexes) from such a scanning probe tip. Such approaches have been discussed in general terms by Drexler.[83,84]

DISCLOSURE OF THE INVENTION—SUMMARY:

The invention produces and combines components comprising structural members which are favorably though not exclusively polymer segments of diverse compositions and well controlled size characteristics, branching and geometry, having one or more affinity groups, which may be of diverse types but are selected or designed to have well controlled specific binding characteristics, and joins multiple components or molecular complexes comprising these and other structural elements hierarchially in the construction of other larger and more complicated molecules, components, molecular complexes or supramolecular structures. According to the methods of the invention, these affinity groups are positioned in a controlled manner, either along or integral to the structures of the molecular building blocks with which they are connected, in a well controlled manner. The position of these affinity groups is generally chosen such that when they bind to their target or complementary affinity groups on other molecular components according to the invention, reactive groups positioned in the involved molecular structures are juxtaposingly aligned such that when conditions are changed to initiate or permit reaction

between said reactive groups, control over which covalent bonds will form, and which bonds will not form, is achieved. Alternatively, these affinity groups are positioned relative to the reactive groups on the respective molecular components such that the relative spatial arrangement of the plural reactive groups is favorable to cross-coupling by the action of a particular cross-linking agent. Such methods of assembly of structures from molecular components are used recursively and hierarchially to obtain complex extended objects and assemblages of such objects. Methods are disclosed which facilitate and comprehensively systematize the construction and production of such complex molecular and supramolecular objects including useful molecular devices. Included within the process and corresponding articles of the invention are structural design methods and corresponding separation methods that facilitate the characterization or verification and purification of the desired products synthesized by the methods of the present invention. Also included within the procedures of the present invention are methods for specifically attaching such objects to microfabricated, microscopic or macroscopic objects, for controlling the motion of objects produced by the process of the invention, and for sensing variations in the structural state of portions of the objects produced by the process of this invention. Some novel objects prepared by these methods and their uses are also disclosed. The process of the invention is applied to the construction of mechanical computational devices, mechanical data storage devices, graphical display devices, the production of probes for use in scanning probe microscopy, scanning probe data storage and scanning probe nanofabrication, and the assembly of microelectronic circuits.

Description of the Figures:

Figure 1 illustrates the construction of an assemblage from molecular components and bridging affinity members. Shown is the assembly of two cruciform molecular components with unique affinity members situated on each terminus into a single assemblage by association of bridging affinity members (505, 506 and 507; and 511, 512, 513 and 514) with their

complementary terminal affinity members (separately). The cruciform components thus decorated with targeted bridging affinities are then mixed, permitting association of bridging affinity 511 (associated with th second molecular component) with the corresponding target terminal affinity member, 501 situated on the first molecular components; this association juxtaposes reactive groups 550 and 557 such that a covalent bond is formed between said reactive groups upon provision of appropriate reagents or physical conditions.

Figure 2 illustrates a fixed digital interlock assembly comprising two intersecting molecular tubular members and two juxtaposed logic lines. Each logic line comprises an alignment member (10) to prevent dissociation from the assembly and to further provide internal alignment of said logic lines with respect to said molecular tubular members during operation. Each logic line comprises a widened region (30 or 45) juxtaposed in the intersection region (27) of said interlock. As depicted, logic line 20 is disposed such that the interlock is closed to translation of the widened region 45 of the other logic line. Logic line 20 is terminally decorated with affinity members 70 and 80, and reactive groups 50, while the other logic line is terminally decorated with affinity members 40 and 60 and reactive groups 50. In this instance, the terminally situated said affinity members (40 and 60; 70 and 80) are each distinct from the others, and may direct the assembly of this fixed digital interlock assembly into larger assemblages.

Figure 3 illustrates the assembly of a fixed digital interlock assemblage from simpler molecular components. Molecular tubule components 650 and 660, on which affinity members (620 and 680; and 630 and 670, respectively) are combined with first logic line 600 comprising widened region 605, first reactive group 603, first alignment member 607 and first terminal affinity group 610, and are of composition and structure such that said molecular tubule components self-assembly onto said first logic line. Bridging affinity member 615 is associated with the resulting assembly, yielding an

assembly with the thus targeted and associated affinity members sequestered from failure product affinity purification. Second alignment member 665 is reacted with the r sulting assembly. A similarly prepared assembly, comprising a second logic line 622 with distinct terminal affinity members (690 and 698) and also comprising two tubular members associated indirectly by bridging affinity member 619 and also two unassociated affinity members 692 and 694, which target the free affinity members of bridging affinity member 615, is combined with the assembly comprising bridging affinity member 615, which directs appropriate association together of the respective molecular components of each assembly into the desired fixed digital interlock assembly

Figure 4 depicts a programmable mobile interlock assembly, which is constrained to move along structural member 220 by macrocycles 210. Displacement of logic line 110 such that widened region 170 is in the intersection region permits closure of the interlock by widened region 180. As shown, this interlock is enabled but open. If closed by the centered presence of 180 in the interlock region, translations of widened region 160 of logic line 100 against widened region 180 would result in corresponding translations of the depicted programmable mobile interlock assembly along structural member 220.

Figure 5 depicts several mechanopotentiators. in (a)-(d), column I shows an illustration of the molecular topologies and column II shows an approximate potential function of displacement. (a) shows a symmetrical entropic spring (tension vectors are subsequently omitted); (b) shows a coulombic mechanopotentiator which relies on the attraction between a first charged functional group situated on the central guest and an oppositely charged second functional group situated on the tubular host, such that a central potential well results; (c) shows a coulombic mechanopotentiator with a different charge distribution and the resulting potential function, showing a distal potential well; (d) shows a third coulombic mechanopotentiator with a charge distribution chosen such that

two terminal potential wells and a central potential barrier results. In part I of (e) there is depicted a more complex mechanopotentiator which may serve as a molecular actuator according to the charge of ionic functions z which are switched according to physical or chemical conditions; part II of (e) shows the potential displacement function according to the polarity of z; alternation of z permits biased translation of the associated macrocycle or tubular member.

Figure 6 shows a strategy for the hierarchial assembly of an array of square-pyramidal molecular tips, for a scanning probe, from molecular components. Distinct affinity groups are denoted by capital letters and complementarity is denoted with a prime (apostrophe). Molecular components with different affinity groups are prepared from the controlled derivatization of functional groups (denoted by squares and diamonds) with different affinity groups. Triangular components are produced by reaction of reactive groups denoted by filled circles with reactive groups denoted by open groups. Bridging affinity members are denoted by letters connected by a short line. Solid phase 1500 permits washing steps which facilitate the hierarchial assembly of dimer tips into the larger array shown. The apical extensions 1480 provides for a high aspect ratio compared to conventional bulk microfabricated probe tips.

Figure 7 depicts a single square-pyramidal molecular probe tip with integrated apical displacement sensing means, showing apex 380 in contact with sample surface 390. Functional groups 300 provide for attachment with a solid probe surface (not shown for clarity). Here, functions 330 may be fluorescent donor moieties and functions 360 may be fluorescent acceptor moieties. As apical rod 310 is displaced from its equilibrium position by contact of apex 380 with sample surface 390, progressively fewer fluorescent acceptors moieties 360 are within coupling distance from donors 330, reducing observed total fluorescent coupling, which is maximum when these acceptors 360 are displaced by flexure of apical rod 310 beyond the coupling boundary, depicted by dashed line 340.

Figure 8 shows an example 1-bit memory cell component implementation suitable for assembly into a three dimensional memory array. This cell provides for data access with either interlock 1090 or interlock 1110, according to the mode of use. The displacement of a bit line, a guest in all interlocks of the cell, represents the data state of the cell. Interlocks 1070a and 1070b provide for the addressed latching of said bit line according to the position of said cell (x and y) within a plane. As shown, 1070a is unlatched (open), while 1070b is latched (closed), such that this cell is enabled for write (translation) in the x but not in the y coordinate. Interlock 1090 provides for the reading of the position of said bit line (here closed by a widened region of said bit line). Mobile interlock 1110 is shown to be closed by the z select line; if interlock 1070b is opened, controlled compliance member 1030 will pull a widened region of this bit line into repulsive contact with the widened region of said z select line closing mobile interlock 1110, the position of which is determined by the displacement of data line 1130. Thus, when said z data line is coupled to said bit line by said z select line, and said bit line is fully unlatched, the displacement of said z data line is written to said bit line. This displacement is then captured by closure of either or both interlocks 1070a and 1070b. Alternatively, when said bit line is latched by one or more interlocks, said bit line may be read by closure of interlock 1110 by said z select and the application of tension to z data line 1130 directed away from controlled compliance member 1030 (for the arrangement shown); the extent of translation of z data line 1130 will be limited by the widened region of the bit line associated with mobile interlock 1110, and the resulting displacement may be sensed or tested by interlocks elsewhere on z data line 1130, or by fluorescent coupling of moieties thereupon with corresponding fluorescent coupling moieties situated on appropriate reference points. Note that closure of either of interlocks 1070a or 1070b latches said bit line into one of two positions (closure latching one of two narrow regions associated with each interlock 1070a and 1070b), such that data interlock 1090 will be either open or closed, representing one or another binary

state of said memory cell. Here, data interlock 1090 provides for reading the binary state of the cell by translation (or impeded translation, when the bit line impedes translation by closing interlock 1090) by the other lin passing through this interlock (1090), i.e. the cross-line.

Figure 9 shows an example copolymer sequencing device, here suited for polynucleotide sequencing. Extended sample single stranded polynucleotide molecule 3200b is assembled into tubule 3070 which with tubule 3200 comprises an interlock. Tubule 3200 hosts guest linear polymer molecule 3010, which is decorated with base-pairing moieties (e.g. guanine 3220) and fluorescent coupling moieties 3010, and which is preferably rigid. Base pairing moieties 3220 of said linear polymer molecule are juxtaposed to the base moieties of sample molecule 3200b. Translation of linear polymer molecule (indicated by 3000) determines extent and type of fluorescent coupling of said fluorescent coupling moieties 3010, such that said extent and type of fluorescent coupling corresponds to different base pairing interactions 3300 between base-pairing moieties 3010 and the base moieties of said sample molecule 3200b. Charged moieties 3030 situated on tubule 3070 determine the energetic barrier to translation of sample molecule 3200b relative to said interlock, permitting step control over translation by application of an appropriate force to sample molecule 3200b relative to said interlock. The lower panel of this figure depicts an adjustable encircling means comprising of molecular components, encircling guest 2070.

Figure 10 shows an example of the two dimensional case of a digitally controlled positioning means comprising linear sliding members 7800, fixed constraining interlocks 7900, sliding interlock 7600, probe 7700 which may for example be an affinity group, a macrocycle, or a molecular tip. Control interlocks 7300 impede or latch widened regions 7500 of linear sliding members 7800. Thus the state of closure of interlocks 7300 controls translations of linear sliding members 7800, and the positional constraints thus imposed provides programmable positional control over translation (under external force) of

the central linear polymers which are the guests of sliding interlock 7600 and are fixedly attached to linear polymer sliding guest members 7800. Two sets of control interlocks 7300 are omitted for clarity; note that in the particular embodiment shown, positioning resolution corresponds to the number of interlocks in a dimension, of which many more than are shown would frequently be desirable.

DISCLOSURE OF THE INVENTION—DETAILED DESCRIPTION:

Unlike chemical syntheses of small molecule compounds aiming to produce specific reactivities, molecular orbital properties or particular geometries and atomic composition, the constraints on the syntheses of larger structures from copolymers are markedly reduced. While physical properties and reactivities are frequently important consideration in the construction of the assemblages and devices of the present invention, the fewer constraints obtaining on the reactive chemistries and particular chemical compounds useful towards the construction of an assemblage or device fulfilling a particular structural or functional goal or use entail that many compositions, specific synthetic pathways and ranges of structures may satisfy predefined structural, functional and physical requirements equivalently.

The versatility, controlled specificity and hierarchial nature of the methods of the present invention thus represent an important paradigmatic shift in the chemical arts, rendering them substantially more amenable to engineering approaches and applications. The methods of the present invention render such larger, highly complex and heterogeneous structures synthetically accessible and provide for systematicity of assembly. Compositional control with nanometer and atomic precision of construction are thus rendered practical, and the methods of the present invention provide for control over structural organization which may be arbitrarily precise to within limits imposed by the properties of the specific structural members used.

This invention produces molecular objects and complexes by combining molecular components (component molecules) having one or more controlled affinity groups and a structural member which may be derived from a lin ar or branched polymer or copolymer molecule of controlled length. These molecular components may further include within their structure one or more reactive groups, positioned in some spatial and configurational relationships to said affinity group or groups. One or more affinity groups that do not participate in the assembly or construction process may also be included for either purification or functional purposes. One or more other functional molecules such as enzymes or other useful molecules, of one or more type, may be assembled onto these components at structurally predetermined locations either before or after the assembly of these components into larger structures.

The procedures for using the elements availed in this invention will be elaborated upon as these elements are described. The invention is a general procedure for designing molecular objects and assemblages with specific structure, spatial configuration and functions from molecular components designed to be consistent with the desired product and the corresponding particular assembly steps. Because of this generality there is quite broad applicability of the process and articles of this invention to a great many structural products and uses thereof. Simpler examples of such assemblages are presented, chosen for clarity and brevity of description, but it will be apparent that the methods disclosed herein may be applied to the production of structures, assemblages and devices with far greater complexity and applicability of use.

It will be understood throughout that measures such as low component or assemblage concentration, gel or matrix entrapment of larger components or assemblages, solid surface, phase or matrix immobilization (at sufficiently low density), or the like will be employed to preclude any significant undesired inter-assemblage or inter-complex associations or reactions. Provided such precautions are observed, each construction will effectively be assembled in isolation from any other

constructions in the same mixture or vessel. Microphases may also be employed to this end.

The term macrocycle will be defined for purposes of the present invention more broadly than in conventional use within the chemical arts; the term macrocycle will, in addition to its conventional applicability, include cyclophanes and cyclic polymers as well as any other ring structure through which any linear segment comprising guest molecule or molecular segment, such as a linear compound as small as propylene, may thread or occupy, where such occupation comprises a structure in which said guest is substantially exposed to solvent or surfaces of compounds separate from said macrocycle. At the other extreme, the term macrocycle may comprehend large cyclic polymers such as circular cosmid or YAC DNA. Thus, the term macrocycle will comprehend any compound through which any other compound or structure may be threaded.

I. MOLECULAR COMPONENTS:

A. Structural Members:

The structural members are polymers which are generally but not necessarily polymers produced by the methods of Zhang and Moore or the numerically precise polymeric synthesis methods described herein, but may also be individual molecules derived from a statistical mixture or polymer molecules. Thus, the extent of these polymeric structural members is defined either numerically or according to a statistical distribution, as may be the location of one or more branchings in such polymeric structural members. No limitations are either specified or required upon the type of polymer employed. Both organic and inorganic[85] polymers may serve as structural members in the molecular components of the invention. The only constraints on the choice of polymer are those dictated by the properties desired in the final product, and those associated with stability during and compatibility with (i.e. stability and desired non-reactivity during) the chemical manipulations performed during the production of components and those performed during the assembly of components into the desired structures or objects.

Three novel methods for the synthesis of polymers of well controlled length are included within this invention.

The first is the combination of the recursive coupling or doubling method disclosed by Zhang and Moore with solid phase synthesis techniques. Contrary to the argument advanced in the disclosure of this method by these authors[86] as to the inapplicability of solid phase techniques to their method, linkage of molecules under synthesis to a solid support need not occupy one of the two ends of the polymer being synthesized (in the case of a linear polymer.) Thus, a polymer may be bound to a solid support and still undergo polymerization at two (or more) termini. Such a linkage between an initial or seed monomer, which is the starting point for such a solid phase synthesis, and a solid support structure, may be accomplished by the incorporation of a functional group in the structure of this initial monomer favorable to the formation of a covalent bond, of a type which is conveniently cleaved at a later time by the appropriate chemical or physical treatment to release the molecule synthesized. The initial monomer is covalently attached by means of this reactive side group to the solid support structure, and then appropriately deprotected and combined with conversely deprotected monomers or macromonomers according to the steps of the solution phase methods of Zhang and Moore. After this coupling reaction has been completed, a fraction of the reaction product may be released from the support, appropriately deprotected, and combined with the differently appropriately deprotected polymer product which was not released from the support. Thus exact doubling of length is achieved, while advantages of solid phase methodology are enjoyed. These advantages include facile separation of products from unreacted reactants and ready separation of the polymer molecules to be used as reactants from the reagents used for deprotection. As with Zhang and Moore's method, precise lengths, sequences of comonomers and branchings may be achieved in combination with solid phase methodologies.

Where longer precise length polymers are desired in fewer recursive cycles than the n cycles required to yield the maximum 2^n monomer length in the method of Zhang and Moore, a 3^n monomeric length may be achieved in n steps, starting from

monomers, by a novel method, with or without the use of solid phase methods, as desired. These methods may be generally termed multiple concurrent coupling, because they exploit controlled growth from two or more termini during the same synthetic step.

Multiple concurrent coupling methods begin with monomers or polymers that may be specifically protected or deprotected at a particular terminus out of two or more termini, where the term terminus refers to any chemical functional group which is susceptible to polymerization. Thus, such a system avails control over polymerization sites by protection and deprotection.

For purposes of description, we may consider a linear polymer and the corresponding diprotected monomers. Monomers are denoted XAY where X and Y are each protecting groups which may be removed by different treatments but which are insensitive to the treatment removing the other protecting group. These methods differ from those of Zhang and Moore by starting with a fully deprotected monomer, A (which will be denoted as A* to indicate that it is a starting monomer and may comprise an affinity group, be immobilized to a solid particle or surface, or otherwise rendered separable), preferably bound to a solid phase or attached to a readily purifiable compound, and adding each type of singly deprotected monomers, XA and AY. Addition to the starting monomer will be step controlled, yielding a trimeric product, XAA*AY. Depending upon other considerations, XA and AY may be added slowly such that they are limiting and thus do not react appreciably with each other, may be added separately in succession, or may be permitted to both react with said starting monomer and to dimerize with each other. In the latter case, dimers may be separated from trimers by physical or chemical means, by affinity separation if said starting monomer included an affinity group, or by washing if said starting monomer was bound to a solid phase. Such trimeric products may be separated if appreciable dimers are present, after which an aliquot of said trimer may be delabeled (i.e. subjected to removal of said affinity group or removal from said solid); said aliquot may be used as a reactant in subsequent coupling steps, in the same way as monomers were

used above. Note that all products retain specifically controllable deprotectability. To continue the example, after further aliquoting of said unlabeled trimer, deprotections may be performed to yield XAAA and AAAY, which are combined with the fully deprotected (but not delabeled) AA*A species, yielding XAAAAA*AAAAY. As with the methods of Zhang and Moore, control may be exerted over branching and comonomer sequence composition. Specific deprotection provides for the synthesis of asymmetric products.

Multiple concurrent coupling may also avail a modification of the alternation of generation techniques developed for dendrimer synthesis. Here, consider a polymerization active comonomer QBQ which polymerizes with monomers PBP by P-Q bond formation. Adopting the same notation above for labeling, second starting monomer PB*P is exposed to QBQ, yielding only a second trimer QBQPB*PQBQ and no side products. Said second trimer is then exposed to complementary third trimers, produced similarly, of composition PBPQBQPBP, yielding only nonamer PBPQBQPBPQBQPB*PQBQPBPQBQPBP. Such alternation of generation multiple concurrent coupling methods, therefore, may avail two parallel syntheses which start from one or the other comonomer type, to yield two parallel reaction courses differing, for example, only in the terminal reactive functional groups at any particular step. Thus, with appropriate polymerization chemistries, no protection or deprotection is necessary. Addition of branched components may permit control over branching. These methods may also effect control over a sequence of comonomers. Note, however, that these methods yield only either symmetrical products or mixtures of products from random additions during any step where more than one species may be added. This will be adequate in many circumstances. It should also be observed that the P-Q bond notation need not refer to a structural polarity, only a reactivity. For example, P may represent some group of atoms ultimately yielding an -O-, and Q may represent some group of atoms ultimately representing $P(O)_2O-$ such that P-Q denotes $-O-P(O)_2O-$, which is symmetrical, and would be the case for polynucleotides synthesized in such a way; in this case, polymerization does not orient a polarity but monomers and

multimers do, and this polarity must be maintained, if desired, during synthesis by control over reagent addition and reactive terminal functionality. Further, a starting "monomer" may be chosen so as to be cleavable by some convenient chemical or physical treatment, and in these cases the symmetry of intermediate products is effectively a consequence of terminal protection by dimerization; in such cases, asymmetrical products may be obtained after cleavage.

Not also, that alternation of generation variants of multiple concurrent coupling methods need not necessarily require the two parallel reaction courses described above, but may instead rely upon cross-linking groups. For example, adopting the notation from above, third trimer QBQPBPQBQ may be reacted with a cross-linking agent, for simplicity here the monomer PBP, to yield first pentamer PBPQBQPBPQBQPBP. Said pentamer may then be exposed to said third trimer to yield an 11-mer, QBQPBPQBQPBPQBQPBPQBQPBPQBQPBPQBQ. As with the above methods, control over reactants added permits control over branching and comonomer sequence. Asymmetric products may again be obtained by sequence specific cleavage.

Note that multiple concurrent coupling methods may be conveniently used to effect alternations of polarity for polymers comprising asymmetric monomers.

For purposes of comparison, syntheses yielding precise length linear polymers by doubling methods will yield a maximum of 64 monomer length after 6 cycles, while tripling yields 729 monomer length polymers in the same number of steps without deprotection steps, and tripling with symmetry breaking central cleavage yields 364 or 364 monomer length polymers, depending on the nature of the starting "monomer".

For purposes of this invention, controlled length need not strictly mean a numerically precise length of polymers. It may, for instance, be desirable to produce an ensemble of objects where some or all of the structural members are together described by a distribution of lengths rather than as being of one identical length. This may be desirable if one intends to experimentally determine an optimal component size rapidly, or if one wishes to produce a mixture with ensemble

properties. One way of achieving this is to use the statistical mixtures of polymer molecules produced by conventional methods of polymer chemistry. Greater control over the distribution of lengths of polymer molecules may be achieved by coupling a distribution of oligomers or polymers to a quantity of precise length polymers produced by the above methods. This is to say that a distribution of lengths is obtained by adding one or more initial mixtures of polymers with some distributions of lengths to a quantity of polymer molecules with an exact length. Thus, complex multimodal distributions of lengths may be obtained in a straightforward manner. By using distributions of lengths of the structural members included in component molecules, one may for instance obtain structures that permit rapid determination of an optimal component length.

It should be noted that in many of the structures whose production by the methods of this invention is envisioned, rigid polymers such as those described above and in other related art are preferred compositions for the structural members presently described. Other preferred compositions include: dendrimers with controlled distributions of chemical functions on their outer surface, synthesized by convergent methodologies[87]; polyfullerenes[88] and polymers of adducts[89] of fullerenes; polycyclodextrin tubules[90]; polyparaphenylenes and their derivatives; ladder polymers; ladder spyropolymers; elastiomers; colloids; and microfabricated structures.

Other compounds, materials or solids may be used as structural members, as a particular design its desired functional characteristics may dictate. Among these are microfabricated solid structures, crystals and crystallites, nanocrystallites and the like, derivatized with affinity groups by prior art methods or the methods described below.

1. Controlled Compliance Members and Energy Transducing Structural Members:

In addition to the construction of controlled molecular structures, it is possible to produce assemblages which store

energy and which are capable of transducing (transforming or converting) one form of energy to another. Various categories of molecules an molecular segments have elastic and other energy transducing properties making them useful components as part of molecular components or assemblages of the present invention. Members comprising these elastic or energy transducing members may be referred to as controlled compliance members or controlled compliance components.

These controlled compliance components may be used to transduce energy needed to perform device operations, i.e. as actuators, or to effect changes in position or compliance property of one portion of one component or assemblage with respect to another part of said component or said assemblage or another. Complex arrangements of one or more similar or distinct controlled compliance members in molecular components or assemblages of the present invention may be constructed to obtain arbitrary desired compliance relationships between two points in the structure of said molecular components or assemblages. A simple analogy for the derivation of desired compliance properties from a fixed set (basis set) of compliance components is the capacitance which results from parallel and series arrangements of capacitors, or the spring constants which result from any complex parallel and series arrangement of springs.

Where said changes in position or compliance property are responsive to some external stimuli, said controlled compliance components may be used as sensors. In particular, sensors of this type transduce sensed phenomena and variations thereof, such as temperature, solution composition, light, electrical field, etc., to changes in position or compliance properties. Thus, sensing and actuation may, as desired, be accomplished by the same components, individually or in tandem.

a. Elastic Members:

Mechanical devices of macroscopic size often utilize elastic energy storing members (e.g. springs, cantilevers.) Molecular assemblages capable of performing controllable mechanical operations or transformations may similarly be advantageously designed with structural members that display various modes of

elasticity. As in the macroscopic case, there are a great many ways to physically implement elastic components. The relevant feature of these members is some characteristic response (which may vary according to other conditions) between extension, compression or displacement from a relaxed configuration and the energy stored in the thus non-relaxed configuration of the elastic member.

A simple case, consisting of a single-bonded linear polymer chain (e.g. polyethylene) covalently bound at its ends to other molecular mechanical components, will display tensile spring-like characteristics for a characteristic range of applied tensile forces and corresponding displacements. This elasticity consists primarily in the increase in the angle between two atoms bonded to the same central atom according to applied linear or tangential forces; linear stretching of bonds may also contribute to a lesser degree to said elasticity. These properties are readily modeled by various molecular modeling algorithms and software packages.[91]

The structures of various molecules and macromolecules suggest these as candidates for use as energy storing or energy transducing components in molecular devices. The compound helicene[92], has been suggested as nanoscale springs[93]. In particular, both linear and comb (i.e. parallel rather than series arrangement) macropolymers of these may yield springs with a desired spring constant.

b. Energy transducing elastiomeric members:

Elastiomeric compounds, whether peptide based, such as those described in the prior art[94], or of synthetic polymeric makeup such as previously described[95], or of distinct classes of polymeric compounds exhibiting similar phenomena, may be employed as energy storing and energy transducing structural members in the components and assemblages of the present invention. Further, these compounds may be incorporated into the molecular components or assemblages of the present invention not only for energy storage and transduction, but also to exert mechanical control over the mechanical operations or transformations of such assemblages or the devices produced therefrom. Incorporation of elastiomers responsive to changes

in some physical parameter or condition, where said response produces a first change in the conformation or configuration of said elastiomer and where said first change in turn produces a second change in the position of assemblages or portions thereof such that some operation is activated, prevented or modulated according to the freedom or restraint of motions thus accomplished. Such parametric control may be understood as the basis of sensing functions, which may further be associated, however directly or indirectly, with motive effectuation. These components are thus of particular interest with respect to applications involving responsiveness or adaptability of molecular devices to various physical conditions or stimuli.

Other molecules or molecular complexes may fulfill the same functional role for interconversion of potential and mechanical energy. Any pair of extended macromolecules which associate with each other over a significant length in a well ordered manner may be incorporated as structural members into molecular assemblages. These may then be mechanically pulled apart or dissociated by physical changes. Such dissociation processes will impart free energy to said extended macromolecules. The reassociation of said extended macromolecules will yield free energy, which may be harnessed to perform mechanical work on other parts of the molecular device or assemblage of which they are part, provided said assemblage is appropriately designed to avail said mechanical work. An example of association energy to work conversion, by polynucleotides, has recently been shown[96] for short molecules, though not identified as useful in this regard. These results may be generalized to longer polynucleotide duplexes, such that polynucleotide length need not pose a limitation on the quantity of energy which may be stored, or the mechanical work which may in turn be performed at the device level during one energy cycle. Thus, two complementary DNA strands may be attached to separate molecular components, such that renaturation of said two complementary DNA strands into a duplex double stranded DNA molecule will perform work on one of said molecular components with respect to the other. The energy cycle of such a system will comprise a denaturation step which stores energy in said system by denaturing said complementary DNA strands, and a renaturation

step during which the free-energy of renaturation causes motions of components of said system, possibly against some force gradient.

Such structural members may therefore convert and store energy at the molecular device level.

Note also that said extended associating macromolecules may be employed to impart strain to points of attachment of said extended associating macromolecules to other molecular components or assemblages thereof, such that self-alignment, specified by the geometry of attachment of said extended associating macromolecules, may be induced in a temporally controlled and predetermined manner against strain forces. The stress-strain relationship will determine requirements for the energy to displacement relationship of the energy storing molecules, and their arrangements. For example, several such molecules may operate in tandem on some fixed point to impart more energy per unit displacement than one such molecule may be capable of imparting.

c. Entropic Springs:

K.E. Drexler has described nanoscale structures obeying potentially useful force-displacement relations, with corresponding energy-displacement properties. Idealized systems of the type described were referred to as entropic springs, and are envisioned as a nanoscale piston enclosing an ideal gas, consisting of one or more atoms of a noble element. Such a system obeys the ideal gas law and the corresponding work-energy relations of classical thermodynamics. Thus, displacements of said piston reducing the volume of the system, against the pressure exerted by said one or more atoms of a noble gas lead to increases in internal energy of said gas. Conversely, permitting collisions of said one or more atoms of a noble gas to cause displacements of said piston reduce the internal energy of said gas, converting said internal energy to work done on said piston. A particularly desirable feature of such a hypothetical system is that geometrical parameters may be varied to obtain desired ranges of force-displacement and energy-displacement relations.

A molecular system may approximate such an idealized system with similar advantages. A first linear polymer molecule serves as a guest for inclusion into the interior of plural macrocycles, as in several rotaxane compounds which have previously been produced. Note that for purposes of the present disclosure, the term rotaxane, wherever appropriate and not otherwise indicated, will include concatenanes; concatenanes share the topological feature of a linear segment or chain passing through a circular structure. A first terminus of said first linear polymer molecule is functionalized with a large steric group which will not admit threading or passage of said macrocycles. Said steric group will preferably be in communication with a first reactive chemical group or a first controlled affinity group. The second terminus of said first linear polymer molecules contains a second reactive chemical group suitable for the addition of further molecular structure. One or more macrocycles may either be self-assembled onto said first linear polymer molecule or similar rotaxane compounds not produced by self-assembly may be formed by statistical means[97].

After assembly of said macrocycles onto said first linear polymer molecules, a capping component is added to said second terminus of said linear guest polymer molecule to prevent escape of incorporated macrocycles. Said capping component preferably comprises the following, in linear order: a chemical group capable of reacting with said second reactive chemical group on said second terminus of said linear polymer molecule, a sterically hindering group capable of convenient removal to leave a second linear polymer segment of similar or identical composition (though not necessarily length) to that of said first linear polymer molecule, a chemically distinct macrocycle possessing one or more affinity or reactive chemical groups and assembled onto said second linear polymer segment, which is in turn adjacent to a non-removable sterically hindering capping group. Said non-removable sterically hindering capping group may bear a distinct specific affinity group and/or a particular reactive chemical group. Thus, said capping component is a rotaxane having one macrocycle entrapped between said sterically hindering group capable of convenient

removal and said non-removable sterically hindering capping group. The incorporation of exactly one macrocycle may be enforced by statistical control, by geometric constraints imposed by the length of the respective linear guest polymer molecule, or by affinity means; purification by mass or size selective means may select for appropriate reaction products.

After said capping component has been added to the structure of the macrocycle bearing said first linear polymer molecule, said sterically hindering group capable of convenient removal is subjected to appropriate physical or chemical treatments to effect the removal of said sterically hindering group capable of convenient removal. The resulting product is a complex rotaxane "necklace" which may, according to the methods of construction, have distinct affinity and/or reactiv groups at each of the two capped termini of the included linear polymer chain, and one terminal macrocycle which is distinctly labeled by a reactive chemical or affinity group.

Said macrocycles, including said terminal macrocycle, may be chosen so as not to have any significantly energetically favorable interactions with adjacent macrocycles of such a rotaxane necklace under some specified chemical and physical conditions. Further, said macrocycles and said first linear polymer molecule may be chosen to not have any significant variations in energetic stability or interaction energy (i.e. variations smaller than thermal energy) as any one of said macrocycles slides along said linear polymer molecule. In these cases, under conditions which may vary from any used to conduct any self-assembly of said macrocycles onto said first linear polymer molecule, said macrocycles will populate the length of the resulting total linear polymer chain, sliding randomly along said chain with thermal vibrational energy and colliding with each other.

The two capped ends of such a complex may be fastened to other molecular components such that they may be drawn taught with some tension. Further molecular components may be attached to said terminal macrocycle at the respective reactive chemical or affinity groups. Alternatively, fastening of such a complex may occur at only the non-removal sterically hindering capping group and said terminal macrocycle. In

either event, sliding of said terminal macrocycle towards the respectively distal end or said linear polymer chain will result in compression together of the remaining said macrocycles, increasing the respective linear number density and thus the number of random collisions between said macrocycles per unit time. Said increase in the number of random collisions per unit time will increasingly impede further compression of said macrocycles. Thus, a force-displacement relation is effected, with a corresponding energy-displacement relation which is determined in part by the thermal energy partitioned into the sliding motion of said macrocycles along said linear polymer chain. The primary parameter for such an entropic spring component is the linear number density of macrocycles. Another significant parameter is thermal energy, which is related to temperature; such entropic spring components may therefore be used as temperature sensors.

Alternatively, said macrocycles may be of molecular structures chosen to mutually interact with adjacent such said macrocycles of similar or different molecular structures, with energies modulated by solvent, chemical and/or physical conditions, in which case said entropic spring will have controllable properties and may further be employed as a sensor component.

Note that said linear polymer guest molecules may have a complex sequence of comonomers or may comprise coupled segments of different polymeric or copolymeric type.

d. Mechanopotentiators:

Components with molecular parts that move with respect to each other (i.e. supramolecular components) may obey force-displacement and energy-displacement relations other that those resembling Hooke's Law of springs, such as obtains for ideal entropic springs. In particular, complex displacement-potential energy relations, having desired degree and configuration, may be obtained with only slight variations on the structure of a rotaxane or entropic spring.

This will be illustrated with coulombic potentials. Consider a rotaxane comprised of a macrocycle member bearing a first

formal charge, and a linear polymer guest member itself comprising one or more second formal charges, (i.e. said linear polymer guest has one or more charged or ionized atoms within its molecular structure.) Where said first formal charge is of the same polarity as said second formal charges, there will be a repulsive interaction between the said macrocycle member and the charged regions of said linear polymer guest member. Said repulsive interaction effects a potential barrier to motions of said macrocycles relative to said charged regions of said linear polymer guest member. Alternatively, where said first formal charge is of opposite polarity to that of said charged regions of said linear polymer guest molecules, an attractive interaction will result. In this latter case, said repulsive interaction effects a potential well with respect to the motions of said macrocycle relative to said linear polymer guest member.

Other interactions and potentials may equivalently be used, to yield different degrees and distributions of potential energy as a function of macrocycle position along a linear polymer guest member with which it comprises a rotaxane. For example, said linear polymer guest member may comprise one or more segments which interact with the interior of a macrocycle with favorable energy, or alternatively with unfavorable energy. In the former case, said macrocycle will experience potential energy wells when at regions of said linear polymer guest member at which it experiences favorable interaction energies; in the latter case, said macrocycle will experience potential energy barriers when at regions of said linear polymer guest member at which it experiences unfavorable interaction energies.

Van der Waals repulsive and attractive interactions may also be availed to effect a potential energy function of position (for example by providing sterically hindering chemical functional groups along the length of said linear polymer guest member which said macrocycle may encircle, but only with energetically unfavorable conformational changes including changes in bond angles and changes in bond length), as may interactions which depend upon solvation energy and solvation energy alteration (according, for example, to exposure of

regions of said linear polymer guest molecule which are particularly well or particularly poorly solvated in a particular solvent under particular conditions.)

Interactions may be chosen to be sensitive to various changes in physical or chemical conditions, and mechanopotentiators may thus be used as sensing components. In these instances, such sensing components may be used to monitor some physical condition or as data or control inputs to the devices of the present invention.

As with entropic spring components, mechanopotentiators are employed by the methods of the present invention in the assemblages of the present invention by incorporation of one or more affinity and/or reactive groups in the structure of said macrocycle, and of one or more affinity and/or reactive groups in the structure of said linear polymer guest member, such that other molecular components may conveniently be placed in communication with the members of these rotaxane mechanopotentiators, and in particular different molecular components or members thereof or assemblages may be in communication with those members of mechanopotentiators whose relative motions are the object of the potential energy function of these mechanopotentiator components.

Note that said linear polymer guest members may have a complex sequence of comonomers or may comprise coupled segments of different polymeric or copolymeric type. Note also that tubules with complex distributions of charge (of one or two polarities), or other structural features affecting the energies of interaction of said tubules with said linear polymer guest members (which may likewise have complex distributions of charge along their length), may be employed in place of a single said macrocycle. Note further that for coulombic interactions, charges carried by said macrocycles may be held by atoms or functional groups coupled to said macrocycles by linkers (or spacers) of predetermined length, such the distance (particularly of nearest approach) between opposite charges may be controlled, and the respective interaction energy thus varied by structural design. Note finally that said linear polymer guest members may have sterically hindering groups which provide limitations on the

motions of said macrocycles or said tubules relative to said linear polymer guest molecules.

2. Rigid Polymer Structural Members:

As discussed above, various methods have recently been developed to solubilize rigid polymer molecules and to produce derivatives of such compounds that have improved solubility properties. In addition to these methods, the present invention may also utilize a novel method for the production of isolated rigid polymer molecules.

Two factors particular to the object of producing nanoscale molecular devices make these methods feasible. First, the quantity of any structural member generally employed in this invention is quite small by conventional macroscale synthesis standards. Second, precipitation generally depends upon random association processes of large numbers of molecules or many portions of highly extended molecules. Theoretically, precipitation processes are resolved into two steps: nucleation and growth. Nucleation is generally the kinetically limiting step, whereby a minimum cooperativity of association between molecules, related to the surface area of a complex available for further association, is formed. Nucleation generally occurs by random collisions and associations of molecules in solution. Precipitation may thus be prevented by fulfilling two conditions: conducting reactions with a sufficiently low concentration of rigid polymer molecules such that the probability and rate of collisions between such molecules are low, and performing all manipulations of such molecules with implements whose surfaces are well wetted with the respective solvent. Formation of nucleation complexes that lead to precipitation is thus prevented, and the surfaces to which the rigid polymer molecules are exposed present no greater affinity for said molecules than the solvent itself. Thus, molecules generally characterized as insoluble are deprived, under these conditions, of any opportunity to come out of solution. This therefore represents a general solution to the problem of insolubility as that problem may bear on the components of the present invention.

It should also be noted that where different rigid polymer molecules are to be associated together in the assembly of the articles and assemblages of the present invention, care must be taken in designs to ensure that associations mediated by affinity groups are more likely, more rapid and/or more energetically favored than random associations between rigid polymers along their lengths. This may be accomplished in a number of ways including the use of extended controlled affinity groups (described below) with self-aligning action and large association energies, the use of different rigid polymer materials which do not associate well, use of solvent systems or derivatives as described above in the review of related art, the use of complexing agents or hosts (e.g. cyclodextrins) that solubilize said rigid molecules and/or compete for sites of association, the use of rigid polymers with patterned structural variation[98,99], incorporation into said assemblages of components or structures that hinder improper close approach and thus random association of such rigid polymers, and by ensuring through structural design that geometries permitting more than some threshold degree of random association between rigid polymer containing molecular components are avoided.

Where solubilization of rigid polymer molecules is accomplished through the derivatization with disordered or hindering functional groups (e.g. flexible side chains), some or all these solubilizing groups may be connected to the main chain of the rigid polymer structure through bonds or functionalities susceptible to cleavage by some specific chemical treatment. Once assemblages are formed, and geometrical constraints reduce problems of insolubilty or precipitation, susceptible linkages may be cleaved such that undesired solubilizing groups are released. The solubilizing groups which are to be retained or released will be determined by the distribution of susceptibly and non-susceptibly linked solubilizing groups. Thus, solubility of a total assemblage which includes such rigid polymers may be preserved while disordered structures are removed from critical regions or components. Such structural designs and treatments will be

important where solubilizing groups may interfere with desired articulations or relative motions of components.

It should be noted that reactive or catalytic functional groups attached to other components may be employed to cleave said susceptible linkages, and that control over the localization of these reactive or catalytic chemical functional groups may thus control the cleavage of said linkages. In particular, this method permits the sequential removal of susceptible solubilizing groups concurrent with the assembly of inclusion complexes of said solubilized rigid polymers, for example, by including said catalytic chemical groups at the opening or mouth of a molecular tube, such that as the polymer enters the tube, susceptible solubilizing functions are removed. Generally, upon such removal, lower solubility of the rigid polymer will favor the stability of the inclusion complex thus produced. Such methods may advantageously be employed in the construction of molecular gates and interlocks, described below.

3. Supramolecular Wire Structural Members and Cylindrimers:

A modification of the procedures conventionally employed to synthesize dendrimers may be employed to produce cylindrical structural members which may be composed or be further modified to conduct electrons, photons or ions.

In place of the conventional starting monomers used to synthesize dendrimers, cylindrical structures, which may be termed cylindrimers, are synthesized with extended linear or branched linear polymers as starting reactants. The substantially linear regions of said extended linear or branched linear polymers include a high linear density of reactive side chains with which monomers or macromonomers such as those used in conventional dendrimer synthesis may polymerize in a stepwise, a generational, or a self limiting manner. With appropriate branching, accomplished by any of the means conventionally employed to control branch occurrence and branch density in conventional dendrimer synthesis, a densely populated polymer surface with predetermined chemical functional composition may be produced.

In a preferred case, after a sufficient number of generations or a sufficient progression of self-limiting synthesis, functional groups ar added to the resulting dense surfac , and these are then subjected to polyvalent cross-linking agents such that the resulting cylindrimer surface is densely cross-linked. Said polyvalent cross-linking agents may further comprise within their structure distinct protected or unprotected chemical functional groups; in this case, such polyvalent cross-linking agents may be used to derivatize the surface of the resulting cylindrical structure, either by comprising chemical functional groups with desired properties or by presenting targets for the binding or reaction to species with desired properties. For example, said polyvalent cross-linking agents may comprise chemical functional groups to which monomers or macromonomers suitable for polymerization into conductive polymers may be coupled or reacted. A complex resulting from such manipulations thus comprises a cylindrical sheet of conductive polymeric composition.

Alternatively, said densely populated polymer surface with predetermined chemical functional composition may comprise a high surface density of metal chelating functional groups. Metals, preferably polyvalent metals, may then be adsorbed to said surfaces. Layered structures such as those described by Mallouk may by self-assembled onto these structures with monolayer precision control. Electroless plating methodologies may also be used.

Depending on the size and electronic structure (e.g. molecular orbital, Fermi band or semiconducting band and band-gap structure) of the resulting complex, it may behave as a microscale wire, a microscale resistor or a quantum wire, for the case of electron conduction.

Where cylindrical surfaces are derivatized with one or more layers of liquid crystalline or other photoactive compounds (e.g. phthalocyanine), a complex with photon-antenna properties may be produced.

Where such cylindrimers prepared in these ways have variously well crosslinked one or more cylindrical surfaces, they may serve as porous, or alternativ ly as substantially closed micro- or nano-capillary tubes or vessels.

Due to the polymeric basis of this class of compounds and complexes, controlled affinity groups such as those described below may be incorporated at controlled locations within these structures. Such controlled affinity groups will favorably be in communication with these structures via linkers sufficiently rigid and long to ensure that said controlled affinity groups protrude beyond the cylindrical surfaces created by these methods, or are positioned at protruding termini of the central linear polymer used as a starting reactant for cylindrimeric synthesis.

These cylindrimers may favorably comprise within their polymer structure rigid polymers or conductive polymers. Further, cylindrimers or complexes comprising cylindrimers may comprise within their structure organic compounds displaying superconductive properties[100,101] including polymers, charge transfer salt complexes and mono- or multilayers.

Note that cylindrimers may alternatively be synthesized from monomers with appropriate highly branched side chains formed in a manner similar to the methods for producing dendrimers but of a structure that favors a generally planar or discotic configuration. In this alternative, cylindrimer synthesis proceeds from disk-like macromonomers in a longtitudinal manner, and may be performed in a step-wise manner or by any of the various precision length polymer synthesis methods described herein. Cylindrimers synthesized by methods of the latter alternative may be termed longitudinally synthesized cylindrimers. Note further that these two alternatives are not mutually exclusive, and cylindrimers of the present alternative may comprise the central linear polymer of the said linear or branched linear polymers as starting reactants of the former cylindrimer synthesis method.

Note also, that as with dendrimer synthesis, the products of convergent polymerizations may be added to central linear polymer starting reactants or longitudinally synthesized cylindrimers.

4. Tubular polymer structural members:

Tubular structural members may be synthesized from macrocyclic molecules without the use of linear guest polymer molecules by a method which is a special case of the hierarchial methods of the present invention. Here, molecules (which may be regarded as macromonomers) comprising macrocyclic structures derivatized with at least two distinct and/or specifically deprotectable/activatable polymerizable moieties are used. Polymerization is performed either by step-controlled single polymer addition methods or the various precision length hierarchial synthesis methods described herein.

A reaction forming a first covalent bond or bonds between a first distinct, single type of a said polymerizable moieties (or first polymerizable moiety) is permitted or caused to occur. At this stage, the dimer or trimer thus formed resembles a flexible linear polymer with macrocyclic side groups. After said first covalent bond or bonds has been formed, and unused monomers and failure products eliminated as desired, one or more plural further covalent bonds distinct from said first covalent bond or bonds is permitted to form, between second distinct, single type of a said polymerizable moiety, such that said macrocycles thus bonded together may not rotate significantly with respect to each other. In cases where more than two polymerizable moieties are comprised within the monomer structure, polymerizable moieties other than the said first polymerizable moiety may be identical or distinct from each other in reactivity, deprotection and/or activation, provided that where similarities exist between such reactivities, deprotection and/or activation, their geometrical arrangement within or along the structure of said macrocycle, in combination with the constraint imposed by the formation of said first covalent bond between said first polymerizable moieties prevents the formation of any bonds which would yield mis-alignment of said macrocycles from forming products with the desired tubular structure.

Multimers synthesized in this way may be further combined according to the various numerically precise polymer synthesis methods described herein, provided groups of steps forming plural bonds between adjacent macrocycles are performed to

prevent the persistence of fr e polymerization competent chemical functional groups and the formation of cross-polymers or adducts between molecules present in the same solution phase. Thus, the length and sequence of tubular members synthesized by these methods may be controlled, as may the incorporation of reactive groups or controlled affinity groups.

Note that such syntheses may be either solution phase or avail solid phase methodologies.

B. Controlled Molecular Recognition and controlled Affinity Groups:

The term controlled affinity group will refer to any chemical functional group, molecular region or molecular surface region, molecular segment or any portion of a heterogeneous polymer sequence, that binds to any other particular target molecule, chemical functional group, region, segment or sequence with a controlled degree of specificity or selectivity under specified binding conditions. Any example of any of the categories of affinity interactions listed among related art affinity may be utilized in unmodified or modified form. Such a definition also comprehends copolymers with some segments only serving a structural purpose while other segments serve an affinity function according to their sequence of comonomers; this particularly includes copolymers with an identical backbone or main-chain monomer structure but differences in the side groups of different comonomers.

Affinity groups may be similar to those found in nature, or may be entirely synthetic, such as those described by A.P. Bisson et al.[102], which display sequence dependent complementarity and self-assembly by zippering.

A special but important case of binding affinities which may be particularly useful to certain uses of this invention, as dictated by design and fabrication considerations, is the affinity of an enzyme (or ribozyme or abzyme or non-biological catalyst) for one or more of the chemical groups which form part of the substrate molecules. In this instance, with the incorporation of such a catalytic molecule as a controlled affinity group, a respective substrate molecule may be used as the target controlled affinity group, and a covalent bond may

thence be formed by the action of the catalytic molecule. Such covalent bonds may be designed to form b tween particular chemical groups (similarly to the discussion of reactive groups below) according to their locations on molecular components but also as constrained by the location of such catalytic molecules on one of said molecular components. By varying conditions, the time at which a covalent bond is formed may be controlled as desired according to the catalytic activity properties of the enzyme. Specificity for particular chemical groups and portions of the molecules, which may further include specificity for particular segments of copolymer sequence, is a property, subject to choice and control, of the particular catalytic molecule or enzyme used. As with associations that do not involve catalytic molecules, controlled association is determined by the molecular recognition process inherent to catalysis. A class of synthetic catalysts displaying such molecular recognition processes, consisting of reaction templates, has been described by T.R. Kelly et al.,[103]; such synthetic catalysts may readily be availed as affinity groups and as functional within the present invention, and more complex versions of these may further be constructed by the methods of the present invention.

The evolutionary methods discussed below may be employed to produce catalytic molecules with modified or altered specificity or modified or altered responsiveness to physical or chemical parameters which may be controlled. An attractive example of a catalytic molecule which may be employed to form covalent links between molecular components is provided by ribozymes, including those which have been artificially modified to ligate polynucleotides together, including cases where such modification consists in in-vitro molecular evolutionary techniques.[104] Here the specificity of the catalytic molecule for a substrate molecule may be designed by well known base pairing rules. Another important example of biologically derived catalyst is the modified peptide ligase recently described by D.Y. Jackson et al.,.[105]

Other classes of affinity groups which may notably be availed by the present invention include: enzyme mimics (which have

additional catalytic properties),[106] diversomer[107] peptidomimetics,[108] and sulfonomaido-pseudopeptides.[109] Modified macrocycles such as the cyclodextrins displaying molecular recognition of specific features of nucleotides described by A.V. Eliseev and H.J. Schneider,[110] or those comprising photochemical antennae described by L. Jullien, J.-M. Lehn et al.[111], or tubular complexes derived therefrom, may additionally be used as affinity groups within the present invention. Other previously described supramolecular complexes, as well as any other molecules displaying molecular recognition may similarly be useful.

While a high degree of binding specificity or selectivity is frequently desirable, there are many circumstances in which it is desirable to relax the specificity which an affinity group will have for potential target molecule affinity groups. In such cases, a first affinity group may be capable of association with any one of a number of second affinity groups which are similar to each other but not identical. This may be termed relaxation of specificity, relaxation of selectivity, or cross-reactivity, and is a characteristic of, for example, so-called degenerate oligonucleotides and of many antibodies. In this way, components which have a general affinity towards a subset of the affinity groups on a category of other component structures may serve as general purpose components or components with flexibility of use while still retaining the advantages derived from binding specificity. The specificity, selectivity or strength of binding (quantified in chemical arts as an association constant or binding affinity constant) of the controlled affinity groups utilized in this invention may further be of such a nature that it changes in response to changes in binding conditions. Such conditions include any physical parameters such as solvent type, combinations of solvents, temperature, ionic strength or ionic composition of the binding reaction solution, chemical reagents or solutes, modifying agents (e.g. mercaptoethanol if there are any cysteine containing polypeptides or other disulfide linkages present), or pH (i.e. acidity or alkalinity). Thus, control refers to the predetermination of any combination of: the

targets which an affinity group will bind to, the strength of binding, the s lectivity and respective strength of binding to similar but non-identical target molecules or target affinity groups; and also to the responsiveness of any of these categories of control to controllable changes in binding conditions.

Particularly for smaller affinity groups, it may be desirable to restrict the conformational degrees of freedom of at least one of the affinities which is a partner in some association of two or more affinities. Where affinity groups are small, linear functions, there are generally fewer conformational constraints, and hence more degrees of freedom and allowable conformations. Only a subset of these allowable conformations will be productive with respect to affinity pairing. For polynucleotides, this does not pose a problem because the hybridization process orders the associating molecules. For polypeptides and other flexible-backbone copolymers, this may be a problem of varying severity. This difficulty may be addressed by the use of affinity groups with modified backbones, modified intramolecular connectivity (e.g. cyclization, polycyclization) or modified backbones such that the accessible conformational states are reduced, and consequently a better defined conformational distribution will be available for binding during a greater proportion of time. Generally, however, it will not be advantageous for all associating partners to have drastically reduced degrees of conformational freedom as this will reduce or preclude the opportunity for cooperative conformational transitions to contribute to binding energies.

The communication or physical linkage of affinity groups to the remainder of the component whose later assembly together with other components they direct may, in particular, be chosen so as to permit removal of all of the affinity groups or some subset of affinity groups on a structure, from either intermediate structures of construction or the final objects produced, by the appropriate physical or chemical treatment after the desired associations or covalent bonds specified by

said affinity groups have been formed. Such treatments include ordinary chemical reagents but may also includ catalytic treatments, treatments with biological or biologically derived enzymes including natural or modified ribozymes; those in the latter alternative may perform the removal with a high degree of selectivity, in a manner that is amenable to predetermination or design.

1. AFFINITY GROUPS OBTAINED BY IN-VITRO EVOLUTIONARY METHODS:

As described in the background of this invention, one important method by which molecules with specific affinities may be obtained involves the selection of molecules from a large random library of oligomeric or polymeric molecules, and may further involve biological amplification, mutation and determination steps. The present invention may utilize affinity molecules obtained by such techniques. Henceforth there have been no reports of experiments with the goal of obtaining affinities with binding strength or degree of specificity that are modulated by binding conditions. For the purposes of this invention, as discussed above, it is particularly desirable to be able to readily obtain affinities whose binding properties are susceptible to desired, predetermined modulation by the variation of physical and/or chemical parameters of binding conditions. This may be accomplished by designing selection procedures more complex than the panning method of G.P. Smith or the SELEX method of Tuerk and Gold.

Rather than designing a selection regime that demands only binding to one target (usually a ligand or a receptor) with a particular minimum strength and increasing competitive binding during subsequent rounds of selection, selection regimes may be designed to select for both desired binding properties and desired types and degrees of modulation of these binding properties by changes in binding conditions. For example, existing methods may be used to obtain, from a large library of compounds, a much smaller ensemble of molecules with some minimum binding affinity and specificity for a target molecule. From the ensemble of specificities thus obtained, one may vary

binding conditions to select release of a binding interaction. Here, one would wash affinities bound to a solid-phase immobilized target, vary conditions, and collect any eluted affinities. Multiple rounds which may include amplification (where appropriate), mutation (where appropriate) and variations of the selection regime may be used to refine controlled affinity properties of these compounds. Thus, one could construct a selection regime that will select for high affinity to a target under low ionic strength conditions but release of said target at some threshold of ionic strength, while all conditions not according to which selection is not varied are held constant.

Complex modulations (e.g. tight binding to a target under low salt conditions with no affinity under moderate salt conditions and strong binding with relaxed specificity under high salt conditions) may be obtained by multiple rounds and modalities of selection as described above, or by combination of individual affinity groups, each having one of the affinity modes desired, into a complex or polyfunctional group that will accordingly have the desired controlled affinity properties.

It should be noted that modifications of the above selection methods have been used to obtain affinity groups specific for particular faces and particular features of surfaces and solid crystals. These methods may be used to obtain affinity species specific for a particular solid surface or type of region thereupon, which may be coupled to a second one or more distinct controlled affinity groups. Such coupled polyvalent affinity groups may be used either as reagents to decorate some solid surface or region thereof with said second one or more distinct controlled affinity groups, so as to prepare said solid surface or region thereof for addition of targeted molecular components, or to prepare molecular components comprising said polyvalent affinity groups and a nanocrystallite, crystallite, microfabricated solid or other particle.

Said polyvalent affinity groups may or may not comprise reactive groups for reaction with said surface or region thereof, or for reaction with other molecular components. Said

polyvalent affinity groups may or may not comprise the extended controlled affinity groups described below. Said polyvalent affinity groups may be used in the construction of scanning probes by the methods described below.

Note that such selection methods may be applied to biopolymers and related compounds, to artificial affinity molecules, including those described by Bisson et al.,[112] . Further, methods such as those recently described by Rebek et al.[113],[114] may be used with synthetic molecules. The methods described by R.M.J. Liskamp may also be applied.[115]

C. Molecular Motors:

1. Biological molecular motors:

Various biological molecular systems have been identified as molecular motors, i.e. molecules which convert some chemical or physical form of energy to directed mechanical work. Such molecular systems generally comprise some motor molecule and some track molecule, for example myosin and polymerized actin, respectively. In recent years, the work performed by such systems has been examined at the level of individual molecules, particularly with regard to forces exerted and displacements effected in a single cycle.[116] Polynucleotide polymerases, such as DNA polymerases, RNA polymerases, reverse transcriptases or RNA replicases utilize the energy of nucleotide triphosphate hydrolysis to translocate their respective polynucleotide templates during the course of the polymerizations they respectively catalyze.

Since biological molecules may readily be targeted in well controlled and highly specific manners by the methods of the present invention, they may be utilized as members of the components of the present invention. For example, some affinity group, such as an antibody, may be incorporated by the methods of the present invention at a predetermined location along a rigid polymer structural member of a first molecular component. Said some affinity group may be selected to have a tight and regionally specific binding interaction with, for example, a myosin molecule, particularly the distal portion of the tail region. A second molecular component, comprising

actin polymer segments positioned by complementary affinity groups, having a structure which holds said actin polymer segment in an extended configuration, may be positioned in some assemblage in juxtaposition to said first molecular component, such that said myosin molecule may interact with said actin polymer segment, so as to perform work in the presence of ATP and under appropriate chemical and physical conditions. Such biological molecules may be engineered by methods known to those skilled in the relevant biotechnological arts to include, for example, cysteins to facilitate covalent attachments, as desired, to the structural members of those molecular components targeted to said biological molecules. Alternatively, attachments may be formed by chemical or enzymatic ligations forming peptide or phosphodiester bonds between said biological molecules and appropriate chemical groups or moieties of molecular components. Note, of course, that care must be taken to incorporate such biological molecules only after any step that may cause chemical damage or denaturation of said biological molecules, and that biological molecules with high stability are favored over easily denatured molecules.

Other biomolecular complexes, including but not limited to F_0-F_1-ATPase, prokaryotic flagellar motors, dynein, and kinesin, as well as processive exonucleases, may likewise be employed.

2. Synthetic molecular motors:

The separate results of J.F. Stoddart, J. Rebek Jr., and A. Harada may be understood as deriving from the relative stabilities or free energies of separate macromonomeric macrocycles plus substantially linear polymeric pre-guest moieties versus the stability or free-energy of the respective guest-host complexes, particularly as changes are effected of said guest-host complexes. Such energetic relations may be readily exploited in the construction of molecular motors.

As a first example, rotaxanes with macrocyclic members having changeable state will be considered. Note, however, that the alternatives of state changes of moieties or chemical groups along the linear members of said rotaxanes, or state changes

involving chemical groups on both linear members and macrocyclic members, or instead involving changes in solvation conditions and relative solubilities, are equivalent physical-chemical transformations for purposes of the operation of synthetic molecular motors. Note also that any of the physical or chemical changes described by D. Urry[117] for elastiomers may be applied to the effectuation of work by the molecular motor systems described here, despite the topological differences.

a. Linear track molecular motors:

Consider a macrocycle with two or more distinct ionizable chemical groups extending towards the center of said macrocycle. Said ionizable chemical groups will comprise at least one group which is negatively charged or anionic under some predetermined conditions, and at least one group which is positively charged or cationic under different predetermined conditions. The ensemble of said ionizable chemical groups are chosen such that some convenient cyclic modulation of chemical or physical conditions may cycle the state of ionization of said ionizable chemical groups such that the macrocycle interior may undergo a cycle comprising a net negative charge under some conditions of said cycle and a net positive charge under distinct conditions of said cycle. Said macrocycle may include a linear guest molecule which bears some distribution of charges along its length. Said distribution of charges may, as followed in one direction along the length of said linear guest molecules, increase from a low density of positive charges to a high linear density of positive charges, followed by an abrupt change to a low density of negative charges increasing to a high density of negative charges. Note that such a polyionic linear guest molecule may be electrically neutral. The ends of said linear guest molecule may be derivatized with bulky chemical group to prevent escape of threaded macrocycles. Physical or chemical changes effecting alternation of the charge at the interior of said macrocycle, without affecting any of the charges along said linear guest molecule, will effect anisotropic translocation of said macrocycle along said linear guest molecules, including against any force gradient. When said translocation of said macrocycle

energy from said physical or chemical changes is transduced to work performed against said force gradient. Wher counterions need be present, they may be chosen so as to have low or no direct association with the ionic groups included within the structure of such molecular motors, to as to not interfere with the motions of the operations of such a complex.

Note that hydrophilicity and hydrophobicity, and interactions based thereupon and modulated by changes in solvent conditions may similarly drive an analogous molecular motor instead of charge-charge interactions, and that combinations of charge-charge interactions and hydrophobicity/hydrophilicity interaction may be combined within one device.

Note further, that the energies and corresponding probability distribution functions may be modified by placing several identical molecular motor components in communication, such that all macrocycles experience an aggregate energy according to the interactions such an ensemble experiences. By such means, the bipositional shuttles of Stoddart et al., which display only moderate localization of the cyclophane member at even low temperatures may be combined to yield stability sufficient for use at higher temperature. In that particular case, the ends of the linear guest members would be aligned relative to some structure that constrains the motions of said linear guest members relative to each other, and said cyclophane members would similarly be joined together in a manner that constrains their motion relative to each other.

b. Rotary molecular motors:

The prior art methods or the methods of the present invention are used to synthesize tubular members, which are then hierarchially linked together in a parallel arrangement yielding sheet structures. These are appropriately derivatized, by the hierarchial methods of the present invention, with affinity groups and/or reactive groups favorable for orderly association yielding cyclization of said sheet, either by bending of said tubular members along their axis or by bending the linkages holding said tubular members in parallel arrangement. The resulting cylindrical structures may then be used as a shaft of a rotary motor.

Cylindrical structures of this type are constructed by the methods of the present invention to have a predetermined arrangement of chemical functional groups or surfaces which will effect predetermined interactions with the corresponding chemical functional groups or surfaces of members assembled around said shafts.

Where said interactions are not sufficiently asymmetric compared to thermal energy or do not involve the transduction of chemical potential energy to time or condition varying conformational state stabilization, such devices may be used as tortional spring members or tortional mechanopotentiators, which may or may not be periodic or symmetrical in rotation-energy relationships.

Returning to the case of rotary molecular motors, the same interaction potential functions implemented on linear members may here be distributed on the surface of said shaft, possibly with multiple repeats either or both along the circumference or along the height of the cylindrical said shaft, such that a macrocycle through which said shaft passes, preferably in a belt-like manner. By changing the chemically or physically modifiable chemical functional groups capable of energetic interaction, or the conditions affecting the energies of said interaction, the angular displacement of said macrocycle may be stepped, as macrocycles are stepped relative to linear members they accommodate as guests in the linear track motor devices described above.

c. Anisotropy of translocations:

i. Conformational Ratchets:

In direct analogy to macroscopic systems, molecular structures which permit facile motion steps in one direction but drastically impede steps in the opposite direction may be utilized. A simple example is afforded by a macrocycle of some circumference through which a first rigid rod polymer segment has been threaded. Said rigid rod polymer may have V-shaped arms comprised of rigid rod polymer moieties of predetermined length, projecting some predetermined length away. All of said V-shaped arms may be oriented in the same direction relative to one end of said first rigid rod polymer. Said V-shaped arms of

said predetermined length are chosen to be of a length which, according to the angle said arms make with said first rigid rod polymer present a distance between the termini of said arms larger than may be accommodated by said macrocycle. By the application of forces to said macrocycle relative to an opposing and parallel force directed along said rigid rod polymer (and in the direction "pointed to" by said "V" arrangement), said macrocycle may bend said V-shaped arms according to their molecular mechanical properties, such that the distance between the termini of said arms is reduced gradually by the structural constraints imposed by the position of said macrocycle. The length, molecular structure of said V-arms are chosen so as to accommodate such bending without imposing undue stresses on the members of such a complex. Thus, said macrocycle may traverse said rigid rod polymer member with surmountable energetic barriers in one direction. Said surmountable energetic barriers may be predicted by molecular modeling software, and the parameters of macrocycle size, composition, conformational energetic state at operating temperatures (e.g. puckering, bond angles, bond lengths), arm length, composition and attachment to said rigid rod polymer, etc., adjusted to obtain a desired barrier height for translation in the direction of ratchetting. When the direction of the force on said macrocycle is reversed (so that it points in or has components along the direction "pointed to" by said "V" arrangement), said macrocycle will be caught by the intersection of one of said arms with said rigid rod polymer, such that further translation will not be possible. Note that less severe cases are also possible, both with said V-arm arrangement and with other arrangements including mechanopotentiation arrangements, such that "ratchetting" is not absolute but merely consists in a differing facility of translation according to direction of translation.

For example, the distance between said termini of said arms in an unstressed state may be smaller than that which may maximally be accommodated by said macrocycle, but requires non-minimal energetic conformations of said macrocycle such that the barriers to translation are determined mainly by the configurational energy landscape of said macrocycle rather than

bending of said arms, said ratchetting is not necessarily absolute but consists in a strong bias in direction of acceptable translation according to the unfavorability of threading both V-arms through said macrocycle relative to the favorability of becoming caught at the intersection of said arms with said rigid rod polymer member.

Such ratchets may, among other applications, be used in inchworm type arrangements and linear actuator structures. Said rigid rod polymer segments may comprise cyclic structures, such that asymmetric translation of said macrocycle does not yield useless end-products or free said rigid rod polymer members from said macrocycle.

ii. Chemical barriers and oriented components:

As noted above, ratchetting is a special case of asymmetry of work for translation in one direction compared to another. Barriers may thus be any potential function with a position-potential energy function which is asymmetric about its maximum. Thus, larger forces will be required to traverse said barriers in one direction than in the other. These components may thus be constructed in the same or similar ways to mechanopotentiators.

iii. Stability cycling by cyclic reactions:

The relative stability of positional states all of the above energy transducing means of this section has relied on localized energy minima which may be traversed through energetic modifications of potential energy barriers or modification of the structures underlying some of said barriers. In the above cases, energy is provided in a manner which is generally stepped or coordinated in an externally controlled manner. This provides convenient means of external control, which may be implemented by simple chemical manipulations which may be performed with automated instrumentation. The same is true for the various prior art energy transducing members other than biological motors.

In other cases, it is desirable that stepping control be controlled by the molecular device itself, whether

intrinsically, by the energy transducing member itself, or by other components of the same assemblage.

II. BRIDGING AFFINITIES:

Further, it is emphasized that the affinity interactions between two affinity groups need not be direct in this invention. Two particular affinity groups on the molecular components of this invention may be brought together by a third, complex affinity group. Thus components with affinity groups may be associated through an indirect binding which is mediated by one or more other intermediary or bridging affinity groups, which may be polyvalent (i.e. combining two or more regions with possibly different affinity properties) and do not necessarily themselves ultimately contribute structural material to the final object of construction. Such intermediary or bridging affinity groups are particularly useful in this invention because they may serve alone or in combination with multiple other distinct polyvalent intermediary affinity groups as a specific chemical glue that will only hold together the affinity groups on other molecules to which they are targeted, and may be added to reaction mixtures at particular stages of a construction process such that there is temporal coordination in the formation of predetermined connections or bindings. This temporal control is useful, for instance, in producing multiple connections with a particular arrangement relative to each other, for example, by passing tethers between two substructures over some other similar tethers and under yet other tethers.

A high degree of versatility and precision in the construction of objects with predetermined structures, structural composition and configuration can thus be achieved. Thus, such affinity molecules are selected or designed to facilitate control over binding characteristics, binding reversibility and binding specificity or stringency during construction steps by changing reaction conditions. Affinity groups may have structures which are rationally designed (e.g. by methods related to rational drug design and rational protein design), may be obtained operationally through random library

selection and enrichment techniques (such as the evolutionary techniques described above), or by other methods known in the chemical, biochemical and biological arts. Examples of molecules suitable as controlled affinity groups which are incorporated in the larger molecular components of this invention are: polynucleotides or oligonucleotides (including single stranded oligo- or poly-nucleotides, as well as triplex forming duplex oligo- or poly-nucleotides used with single stranded oligo- or poly-nucleotides having appropriate sequences according to triple helix base association), modified polynucleotides or oligonucleotides (including unusual or non-natural nucleoside bases and modified backbone structures such as those of peptide nucleic acids or methylphosphonate oligonucleotides,), peptides or polypeptides, protein fragments (synthetic or naturally occurring), proteins, biological receptor molecules and their ligands, antibody molecules (including monoclonal antibodies, or protein fragments or molecules substantially derived from immunoglobulins), crown ethers, carcerands, self-assembled polymeric binding affinities such as molecularly imprinted polymers, or any other molecule or ensemble of molecules which have characteristic binding properties to some specified group of target molecules.

Further, bridging affinity molecules may rely for molecular recognition on one or more catalytic molecular segments or functions or subunits included within the bridging affinity molecule structure.

A convenient and simple example of an intermediate bridging affinity which recognizes affinity groups incorporated into separate molecular components is streptavidin, which is tetrameric and binds four biotin molecules. Biotin may be included as an affinity group on multiple molecular components where these are to be targeted together. These biotins will be inert towards each other, but will be collocalized upon addition of streptavidin or any homologous binding protein.

Another simple case is presented by single stranded oligo- or poly-nucleotides (bridging polynucleotides) comprising sequences complementary to two or more oligo- or poly-nucleotide affinity groups each situated on a different molecular components, where said two or more oligo- or poly-

nucleotide affinity groups do not have sufficient complementarity to pair with each other; thus, mixing or contact of such a bridging polynucleotide with said two or more molecular components under binding conditions will cause said two or more molecular components to be bound together via said two or more oligo- or poly-nucleotide affinity groups and via the intermediate said bridging polynucleotide. Thus, the sequence of said bridging polynucleotide specifies or programs the collocalization of said two or more oligo- or poly-nucleotide affinity groups on said different molecular components according to the sequence identity of said two or more oligo- or poly-nucleotide affinity groups. Such collocalization may serve, in accordance with the general nature of the present invention, to bring reactive groups occurring within the structure of said molecular components into juxtaposition with predetermined other reactive groups occurring within the structure of said molecular components, such that said molecular components are joined together in a precise predetermined structure with only the desired covalent bonds formed between the correct pairs of said reactive groups. An example of this process is shown in Figure 1, with bridging oligonucleotides (e.g. ssDNA 8-mers) represented by 505, 506, 507, 511, 512, 513 and 51^4, and with reaction between reactive groups 550 and 557 (e.g. thiol (-SH) groups) directed by bridging oligonucleotide 511 bound to the two affinity groups 501 (e.g. ssDNA 4-mers) and occurring under oxidizing conditions, which are preferably effected only after the introduction of bridging oligonucleotide 511.

III. PROTECTING AFFINITIES:

In addition to serving as means for co-localizing regions of molecules to be specifically joined together (either intra- or inter-molecular associations), affinity groups may be employed to selectively prevent such associations. Affinity groups used for this purpose shall be referred to as protecting affinities or blocking affinities. This may be achieved by the addition of one or more affinity groups complementary to one or more of those affinity groups which would associate in the absence of these affinity groups, before the affinity groups which specify

the presently undesired association have the opportunity to associate.

In this protecting mode of use, a first, protecting affinity group, which is not covalently linked to any component structure, is reversibly bound to a second affinity group which is part of a component structure, such that any other affinity group targeted to the same surface of said second affinity group will not find its protected (i.e. concealed, unexposed, blocked or sequestered) target affinity group. The protection afforded by a protecting affinity group consists in the exclusion of any other affinity groups specific for the same target affinity group from the region to which it binds. This exclusion process differs only in temporal sequence and degree from situations of competitive binding (which need not be strictly exclusive in a reaction mixture) of two affinities to one target molecule. Thus, until the protecting affinity group is removed by the appropriate chemical, enzymatic or physical treatments, the protected affinity group will be inert to binding by any other affinity group targeted to it, or at least targeted to the same region of the surface of the target affinity group. The affinity groups on a component molecule or structure may thus addressably have their binding capability towards other components deactivated reversibly. Multiple parameters of control over the order of formation of desired associations may thus be accessed.

To illustrate this affinity protection method, we may consider a first component molecule, the structure of which consists of a polyethylene molecule with two different single stranded oligodeoxynucleic acid sequences covalently joined to its termini. In this example, each of these oligodeoxynucleic acid sequences are specific affinity groups. Each of these oligodeoxynucleic acid sequences differ from the other and the first oligodeoxynucleic acid member is complementary, according to Watson-Crick base pairing rules, to a oligodeoxynucleic acid sequence on a second component molecule. Before the second component molecule is introduced into the same vessel with the first component structure, the first component molecule may be addressably protected at the appropriate oligodeoxynucleic acid member from binding to the complementary oligodeoxynucleic

member on the second component when this second component is later added. This addressability of protection is achieved by using a protecting affinity function which is complementary to the affinity group to be protected, in such a way that its binding prevents the binding of the complementary affinity group whose binding to the first affinity groups is to be prevented. The molecules chosen as protecting affinities are chosen such that there presence may be selectively eliminated. In this example, a protecting affinity may be a single stranded oligomeric sequence of ribonucleic acid, whose protection may be eliminated by, for example, denaturation, hydrolysis in the presence of base, or treatment with the enzyme ribonuclease H. These treatments are chosen so as to leave all affinity groups on components intact (to the extent required by subsequent assembly steps). Note that different types of protecting groups (each type targeted to a different set of target affinities on component structures) may also be chosen so as to be sensitive to, or have binding properties sensitive to particular treatments such that one treatment will only deprotect one set of affinity groups. By these protection methods, components that are to form multiple, overlapping associations, which yield different structures according to the order in which associations are made, may form these associations in a controlled rather than a random manner, such that only the desired product is obtained.

IV. REACTIVE GROUPS AND CROSS-LINKING AGENTS:

By the term reactive group any group of atoms within a molecule that are capable of reacting with other like or different reactive groups to form covalent chemical bonds, according to their respective chemical nature, is meant. The reactivity of a vast number of different chemical functional groups with other chemical functional groups under given conditions are well known within the art of chemistry.

The reactions which ultimately link together two reactive groups on the molecular components of the invention need not only involve the two reactive groups on these components; one or more other molecules, termed a cross-linking agents, which

have two or more reactive groups among which at least two will react with the correspondingly sufficiently co-localized reactive groups of the component molecules, may form an intermediary covalent linkage between both of the reactive groups of the molecular components, which joins appropriately co-localized plural component molecules together into one molecular structure or otherwise forms specified covalent linkages. Cross-linking agents may also be of polymeric, macromonomeric, or macropolymeric composition. Such cross-linking agent molecules used to link together component molecules or structures (which components may not, and in many instances are preferred to not react together without the incorporation of other molecules) may or may not themselves have affinity groups incorporated within or attached to their structure. The plural reactive groups on a cross-linking agent molecule may be similar or identical or entirely distinct from each other, according to the particular reactive chemistries desired and the particular reactive groups to be thus linked together. One or more of these reactive groups may be in a protected state or in an inactivated state (according to the chemistries utilized) at the time which it is introduced into the reaction vessel with the components of the object under construction. Accordingly, deprotection or activation may be effected, by the appropriate techniques of chemical art, at the desired time during construction. Additionally, the structure of a cross-linking agent may be chosen such that desired flexibility and degrees of configurational or conformational freedom between the reactive groups of the molecule is availed, and such that desired relative location and relative orientation of the reactive groups is favored while undesired relative location or orientation are disfavored. These factors will affect the likelihood that the covalent linkages effected through the cross-linking agent occurs with the desired specificity and good efficiency. For instance, where there is uncertainty about the exact spatial distances within a structure under construction, these crosslinking agents (or the linkages of reactive groups to the rest of the respective component molecule) may have more degrees of freedom, such that

linkages between component molecules that are not optimally aligned may nonetheless be joined together covalently.

For clarity, the description of reactive groups above has mainly discussed the localization together of two reactive groups with appropriate reactive chemistries, followed by the single reaction between these. Without departing from the theme of affinity co-localization of reactive groups followed by their reaction together, it is possible to design components, and assemblages of such components, that provide for cascade or chain reactions which polymerize multiple reactive groups. Such covalent component polymerizations, like the linkages formed between reactive groups in the foregoing discussion, may either be direct or may be mediated by cross-linking agents.

It should be noted that the assemblages or constructs of this invention may also employ molecular components having appropriate reactive groups and structural members but lacking affinity groups. In this special case, these molecular components may be regarded as macromonomeric cross-linking agents. Specificity need not be sacrificed with these components in instances where the reactive groups of such an affinity-group-lacking-molecular-component are of particular types and relative spatial constraint, such that the component in question will react only in the desired way with a pre-existing construct. A simple example is that of a component with two distinct reactive groups at each terminus of a rigid polymer of defined length. If one reactive group, capable of reversible reaction, is reacted with the pre-existing construct, and then the second reactive group is permitted to react, followed by a return to conditions that are not permissive to the reaction of the second group with the pre-existing construct, and then the bond formed by the first reactive group is temporarily cleaved, and the construct washed, and the first reactive group permitted to react again with the appropriate reactive group on the construct structure, only the desired bonds to the affinity group lacking molecular component will be formed.

It will be recognized that, where conformations and structural configuration permit, these methods may facilitate

one or more intramolecular cyclizations involving one or more molecular components within some assembly or in isolation. This may be particularly useful in the construction of a diverse range of interlocks according to other aspects of the present invention.

While the full range of covalent chemistry may apply provided that the information contained within affinity groups is not irreversibly lost, a few classes of reactive groups recommend themselves. First among these are sulfhydrils, a pair of which are capable of forming disulfide linkages under oxidizing conditions. This reaction procedes well in aqueous solutions and does not adversely affect the nucleophiles or electrophiles of polynucleic acids nor peptides not containing sulfhydrils.

A second class embraces light initiated bond forming reactions. For affinity groups comprising ssDNA oligonucleotides which bind molecular components together via complementary binding, and which are suitable for the formation of triple helical structures with an appropriate third oligonucleotide, said appropriate third oligonucleotide may be derivatized with psoralen. Said molecular components are bound together, and then said third oligonucleotide modified with said psoralen is permitted to bind to the duplexed oligonucleotide joining said molecular components. The resulting complex is then irradiated with an appropriate source. For an example of triplex directed psoralen crosslinking initiated by irradiation, see C. Giovannangeli, C. Helene, et al., **1992.**[118]

Aqueous photopolymerization is also useful for acetylene functions[119] and cinnamic acid derivatives[120]. In each of these cases, two identical unsaturated functions are juxtaposed by affinity binding. Said unsaturated functions are preferably situated on small flexible linkages to said molecular components such that they are free to adopt conformations favorable to the initiation and completion of bond forming reactions.

In such cases where UV irradiation is used to promote bond formation, where affinity groups comprise polynucleotides, said affinity groups and said polynucleotides preferably do not comprise two adjacent thymine residues, as these may form

cyclobutyl crosslinks upon such irradiation, which is a primary damage mode of DNA under the influence of UV radiation, and which is thus readily avoided.

A third class or reactive groups embraces functional groups which may be selectively caused to react together to form covalent bonds, by the use of catalysts, and especially synthetic catalysts, and most especially biological enzymes or artificial enzymes including ribozymes. A first example is provided by amino acid reactive groups joined by the action of peptidyl ligases. A second example is the ligation of polyribonucleotide functions (which might be, but need not be separate from a controlled affinity group) by RNA ligase ribozymes[121] (which themselves might be, but need not be, separate from a controlled affinity group,) where the hydroxyl donor for such a catalytic reaction resides on a different molecular component than the 5' triphosphate acceptor (i.e. the alpha phosphate of a 5' ribonucleotide triphosphate.) A third example is the esterification of a primary aliphatic alcohol with a terminal aliphatic carboxylic acid function by a lipase, in aqueous, or organic[122] media.

V. CAPPING OR ELIMINATION OF FAILURE PRODUCTS:

In the synthesis of even moderately complex molecules with known art methods it is often advantageous to introduce synthetic steps that modify reactive groups that did not successfully undergo some desired reaction step and whose further later reaction would compromise the homogeneity of the desired product. Such measures are availed in automated oligonucleotide synthesis to prevent any further nucleotide addition to molecules that failed to incorporate a nucleotide during the previous addition cycle. For purposes of the present invention, the ability to prevent further assembly of objects that failed to incorporate some component or make all desired associations or make all covalent linkages is likewise advantageous. The novelty of the assembly methods of the present invention demands similarly novel methods to accomplish such a capping function.

In a first case, that of affinity association failure products, capping consists in binding the first affinity groups

which failed to bind to a second target molecule during the preceding steps to a capping module comprising a third affinity group with appropriate specificity and a fourth functional group that facilitates some means of separation or purification. This fourth functional group may be a distinct affinity group which is then selectively retained (with the failure product to which it is now associated) during some affinity purification step; alternatively, for objects that will be electrically neutral at some stage, this fourth functional group may be ionic, permitting electrophoretic separation, electrostatic separation, precipitation, extraction or any other appropriate manipulation; other purifiable fourth functional groups may readily be envisaged by those skilled in the chemical arts. Note also that this fourth functional group may further be or include as a substituent a dye molecule that facilitates fluorescent sorting, or separately facilitates spectrometric quantitation.

In a second case, that of failure to form a covalent bond between reactive groups, there are two possible capping methods. The first is to simply expose the products of a preceding covalent bond formation step to a reagent that will react with all unreacted reactive groups. In this instance, there can be no reactive groups that are to be spared, i.e. no unprotected reactive groups to be utilized at some later step. The second capping method relies on the addressability of reactive groups that are in a controlled spatial relationship with an affinity group in the assembly. In this instance, a capping module comprising a fifth affinity group with appropriate controlled specificity and a reactive group suitable as a capping reagent. This capping reagent group will be reactive to some unreacted reactive group of the assembly failure product when in one state (e.g. deprotected or activated) or under specified physical or chemical conditions but otherwise inert. The capping module is first associated via targeted affinity interactions of the third affinity group with the appropriate affinity group or groups of the assemblage, and then the capping reagent is deprotected or activated or conditions are changed to permit the capping reaction to occur. Finally, at some later stage which is

determined by the specific design of the objects under construction, affinity or other purification steps are employed to separate failure products from successfully formed products.

Both types of capping modules may be specifically labeled with a unique fourth functional group comprising a unique affinity group and/or a unique photolabel (e.g. dye molecule) to permit both categorization and quantitation of failure products. Thus, where several distinct capping steps are availed during a construction process, it is possible to glean information useful to debugging or troubleshooting the construction process and/or the design of the assemblage.

Note that where capping modules rely on affinity associations, as in the first case and the second instance of the second case, the third affinity group may be degenerate or otherwise posess relaxed specificity whereby it will bind to a well defined set of affinity groups on the assemblage. Further, in the second instance of the second case, where failure products that did not form a covalent linkage are to be addressably targeted, the third affinity group may be targeted to any of the individual affinity groups in a manner that is not hindered by their possible association with other affinity groups which they target (i.e. in a non-competitive manner, to a different region that is not blocked) or to some portion of their associated structure (e.g. as is the case for triple- or quadruple-helix formation of polynucleotides for the third or fourth polynucleotide to bind), as is deemed appropriate to the specific design.

VI. POSITIONING AND BINDING OF FUNCTIONAL COMPONENTS:

Affinity groups may likewise be used to localize within the structures of the objects which are the articles of this invention functional component structures comprising one or more complementary affinity groups and one or more functionally useful molecules (or structures or complexes or particles) such as enzymes, conducting molecules, rectifying molecules, photoactive molecules and complexes, dye molecules, fluorescent molecules, colloids, metallic clusters, microfabricated or microelectronic solid state electronic components,

piezoelectric particles, invar particles, magnetic particles, paramagnetic particles, wires, quantum wires, quantum dots, photon wave-guides, chelators, carcerands, cryptands, cavitands, crown-ethers, calix[n]arenes, electron transfer moieties or proteins, receptors or receptor ligands, antibodies, reverse micelles, liposomes, elastiomers, actuators, zeolites, etc.,. Functional components may include affinity groups for purification or separation purposes, or may include labels such as fluorescent labels to aid in separation of objects with all desired components (for instance by fluorescence automated sorting such as that used to sort mammalian cells in the art of cell biology.) Such diverse components may thus be programmably localized in a well controlled manner at desired locations on or in the components of this invention or the completed assemblies or objects produced thereby and therefrom. These functional components may either be covalently joined to the structure of the assembly during subsequent steps of the procedure or joined to the structure only by the affinity association which initially localizes them within or on the structure. If covalent linkages between functional components and the molecular components of this invention (or their assemblages) are to be made, then both the appropriate molecular components and the functional components must include reactive groups capable either of being reacted with each other to form a covalent bond or of being covalently cross-linked by a cross-linking agent. These reactive groups must be positioned relative to the affinity groups which will associate the components together such that when affinity mediated association occurs, a relative position and orientation of the reactive groups with respect to each other, favorable to the desired reaction, will result.

VII. AFFINITY PURIFICATION:

Likewise, each component may include one or more affinity groups that serves as an affinity label for purposes of purifying assemblies or assembly intermediates with various combinations of components, as desired. Like all other affinity groups of the present invention, these may be designed

to be removable from the object of construction, or may be left in the final construct as desired.

Alternatively, affinity purification may take advantage of any affinity group or any portion of the surface of an affinity group which is otherwise employed in the construction of the articles of the present invention.

Separation of molecules or complexes may therefore proceed according to the state of some affinity group with respect to the respective target of said some affinity group. By such means, those affinity groups, and hence the molecules or complexes to which they are attached, which have not bound to their targets, may selectively be purified by any second affinity group which binds sufficiently tightly for retention but will not bind to said some affinity group when said some affinity group is bound by the respective said target. Thus, unsuccessfully bound components may be separated from desired product structures, as desired before the formation of any covalent bonds in the respective stage of construction, and optionally recycled or characterized to determine the reason for lack of binding, if unexpected.

Alternatively, affinity purification may be chosen to selectively bind those structures which include some surface comprised of determinants or portions of two or more components. This instance of affinity purification will retain those structures which include successfully targeted affinity groups. For instance, two components may comprise complementary single-stranded oligonucleotide-based affinity groups. Binding of said single-stranded oligonucleotide-based affinity groups will yield oligonucleotides which are double-stranded or duplex along some fraction of their length. Such duplex regions may be designed to facilitate the formation of, for example, triple helices when presented with a single-stranded oligonucleotide of appropriate complementary sequence. Said single-stranded oligonucleotide of appropriate complementary sequence may therefore be bound to a surface or matrix and used as a specific affinity purification reagent to bind only double-helical oligonucleotides of appropriately complementary sequence. Such purifications will be carried out under appropriate physical and chemical conditions, as will be

obvious to those skilled in the relevant arts. For purifications in which it is desired to recover any retained components or structures, conditions of the resolution medium or mixture may then be adjusted, after transport of unretained fractions (e.g. by washing or gradients) is completed, to release retained species.

Alternatively, instead of relying on a solid surface or matrix, affinity groups may be labeled with appropriate labels (e.g. second affinity labels, photolabels, fluorescent dye moieties, etc.,) mixed and permitted to bind with some mixture of structures and components, and then separated in fluid phase according to said appropriate labels, in a manner closely analogous to fluorescence automated cell sorting.

Note that affinity groups or molecules used as affinity purification reagents may be designed or otherwise derived by any of the methods which affinity groups used in the components of the present invention are produced or derived. For example, some first affinity groups to be incorporated at some location of a molecular component may be separated into aliquots, the contents of one such aliquot may be bound to a surface, and a library of second affinity groups (e.g. an epitope library, previously depleted for binding activities which bind the first-affinity-group-unmodified surface, i.e. to eliminate non-specific binding activities) exposed to said surface, and any second affinity groups retained by the first-affinity-group-modified surface are recovered. Said second affinity groups retained are thus known bind specifically to said first affinity groups, under those conditions which were used for the binding exposure, and are also known to dissociate from said first affinity groups under those conditions used for recovery of said second affinity groups retained. Additional rounds of preselection may be used, as with *in vitro* molecular evolution, to obtain affinity purification reagents with the desired ranges of specificity and ranges of responsiveness to changes in conditions.

VIII. SOLID PHASE METHODOLOGY:

This invention also combines, as desired, the use of solid phase supports with the other methods described herein. Solid

phase synthesis methodologi s are useful because the attachment of some component or assembly of components that is under construction facilitates purification and other handling steps, greatly facilitates the recycling of unused reagents and solvents, and is further useful in the automation of the procedure. Solid phase methodology shall refer to any physical attachment of any of the components or assemblies which are articles of the present invention to any insoluble body or to any matrix media such as those used in chromatography, at any stage during their synthesis or construction. Examples of insoluble bodies include colloids, polymeric beads, magnetic beads, gl ss beads, and controlled porosity glass beads. Examples of matrices include cellulose, polyethylene networks, sepharose, agarose, polyacrylamide, silica matrices, hydroxappatite, diatomaceous soil derived matrices, and any solid surface capable of chemical derivatization including metals and plastics.

IX. HIERARCHIAL ASSEMBLY OF TWO- AND THREE-DIMENSIONAL STRUCTURES FROM MOLECULAR COMPONENTS:

Prominent among the categories of structures or assemblages of technological interest which may be assembled by the methods of the present invention are two- and three-dimensional structures. Approximately two-dimensional structures will be referred to as sheets, and may be unimolecular, may be comprised of two or more interleaved molecular networks, or may be more elaborate molecular complexes. Though it is generally obvious from the teachings of the present invention that three-dimensional structures and assemblages may also be produced according to this invention, particular categories of interest are regular three dimensional lattices, networks and/or arrays. Further, by variations on the methods described in this section, i.e. through th combination of markedly different assemblages at appropriate st ps of hierarchial construction, two- and three-dimensional structures and lattices with compositional and/or structural heterogeneity may be achieved.

To maximize the control over the reactions leading to such extended structures, and to prevent or minimize products which

contain all desired components but do not have all of the desired predetermined connectivity between components, the assembly together of components and the formation of subsets of all of the desired connections to be formed at any given stage of a construction process is conducted in a controlled stepwise manner.

The essential procedure underlying these hierarchial constructions (i.e. each "hierarchial step", not to be confused with associative or synthetic steps) of two- and three-dimensional assemblages will now be described. Note that the assembly step consisting of each hierarchial association may combine two or more individual molecular components, may combine assemblages of such components, or may combine molecular components with assemblages of such components. The term "component" may therefore be replaced in the foregoing description at many points by the term "assemblage". Alternatively, macrocomponents (which will generally be assemblages) may be understood to be comprehended by the term component. Note also that for hierarchial assembly steps, according to the control exerted over the associations (directly analogous to the various means for synthesizing controlled length polymers), multiple alternative constructive combinations are possible: two components may be interconnected to form a larger assemblage; three components may be simultaneously interconnected to form a larger assemblage; or associations analogous to those of conventional polymer chemistry, instead using molecular components or assemblages as macromonomers which are permitted to associate in a manner that does not directly control the number of macromonomers that associate at once (except through stoichiometry, competition, or modulations of reaction rate, etc.) but having formed the specified orienting connections, according to steps otherwise directly analogous to those for direct numerical control, may then be subjected to steps that yield the formation of the correct plural interconnections between each such macromonomer. Other constructive combinations will be possible. Note that the term juxtaposition will comprehend both the strict colocalization of two functional groups (affinity or reactive) and additionally

the imposition of constraints on the relative positions which may be assumed by thus mutually oriented components or assemblages relative to each other, such that while not collocalized at all times or in all possibly configurations, each first reactive or affinity group which is desired to form a connection with a respective second reactive or affinity group on a different component or assemblage (connectively oriented relative to the component or assemblage of which said first reactive or affinity group is a part) may only come in proximity with said respective second reactive or affinity group. Thus, as in the case of strict collocalizing juxtaposition, the broader application of the term will still entail that each member of a pair of sites to form connections will have a greatly enhanced effective concentration for the other member of said pair, and improper pairings are correspondingly simultaneously excluded by the same mechanism.

In essence, two- and three-dimensional structures are formed from smaller, appropriately structured and composed one-, two- or three-dimensional structures by first forming a first orienting connection (affinity or covalent) to constrain the possible collocalization and interaction between all other connecting groups on each such component such that only desired connections have any opportunity to form and all other connections are precluded by geometrical constraints. Multiple orienting connections may be formed as needed or desired. Only after such constraints are imposed by orienting connections are other connections permitted (e.g. by controlling reaction conditions, the presence of cross-linking reagents or bridging affinities, etc.) to form.

A first molecular component is associated with a second molecular component in a targeted manner such that one of the affinity groups on said first component is bound by the targeted affinity group on said second component. These two components are thus bound by one association, such that they have one node or vertex in common. Components are designed such that functionalities which are to associate or react with other functionalities will have good spatial proximity to each other as a result of this first association. This first connection is exploited in the orientation of subsequent

associations and/or covalent bond formations. Said first connection will thus be termed an orienting connection. Subsequent connectivity is thus preorganized before its formation.

In one variation of this procedure, said first connection may occur at one terminus of the two molecular components, and a second orienting connection is formed either an appreciable distance from said first connection towards a second terminus of said first or said second component, or at said second terminus. Said second orienting connection eliminates at least one rotational degree of freedom about said first orienting connection. Subsequently, further orienting connections may be formed, preferably in a controlled manner one at a time, until there are arbitrarily many constraints (at least one posed by each of said orienting connections) on any otherwise available degrees of freedom between said components (including vibrations). Thus, a large number of reactive (or affinity) groups (including undifferentiated affinity groups) may be juxtaposed in a uniquely desired configuration such that desired connectivity is greatly favored and all other connectivities are greatly disfavored. Where reactive groups are such that covalent bonds do not form spontaneously upon juxtaposition, crosslinking agents or other means of control over reactivity are introduced after all orienting connections have been formed.

In a second variation of this procedure, which will be adequate for the correct interconnection of rigid or low-flexibility components or assemblages, a first orienting connection is formed between two mutually targeted components. Due to the rigidity constraints inherent in these components, one swivel joint (even with completely unrestricted rotation) will be sufficient to order all other affinities or reactive groups of the involved components with respect to each other such that desired pairwise juxtapositions occur and all other proximities are eliminated or disfavored.

By these methods, two or three linear molecular components may be combined to form small two-dimensional ribbons or sheets. With appropriate design of the starting molecular components, two or three sheets composed of two or more

molecular components may be combined together (again by first forming an orienting connection at one well defined point on each precursor followed by forming associations or bonds at other sites, reactive groups or affinity groups) to form well ordered larger sheets composed of controlled numbers of molecular components. The products of each such steps may be combined recursively, with, for instance, successive alternations in complementarity of affinity groups, or appropriate modifications such as expansion or deblocking of affinity groups to ensure unique orderly controlled targeting and numerical control as well as precise compositional control. Thus, in a manner analogous to the synthesis of precise length and sequence linear polymers, two-dimensional sheets may be synthesized so as to have precise compositional configuration and precise dimensions, including precisely localized affinity, reactive and catalytic groups, and functional components.

Such approximately two dimensional sheets may comprise a dense distribution of metal chelating chemical functional groups, to which metallic ions may be bound from solution, to produce quantum wires and quantum sheets. Alternatively, quantum wires or quantum sheets may be produced by the electroless plating of such approximately two dimensional sheets.

Such approximately two dimensional sheets with precise configurations of controlled affinity groups and reactive groups may likewise be combined together, preferably with other such sheets having complementary configurations of targeted affinity and/or appropriately reactive groups, to produce three dimensional structures with highly controlled structure and composition, including highly controlled configurations of catalytic groups and/or functional components. As in the formation of sheets, larger three-dimensional structures may be built up recursively from smaller three-dimensional structures (e.g. composed of a smaller number of sheets) with numerical and compositional controls. The formation of a single orienting association in a first step, optionally followed by a second and possibly a third orienting connection, and finally followed by formation of all remaining desired connection

between juxtaposed reactive or affinity groups will again favor high yields of desired products with low failure products.

Thus, by exerting control over the composition, structure and utilization of molecular components, sheets made therefrom, and over the combination of plural sheets into three dimensional structures, arbitrarily precise control over the composition of the resultant three dimensional assemblage structure may be achieved to the extent consistent with chemical principles and stability.

In cases where it is desirable to rely less on orientation by affinity groups, orienting connections may be formed between two components or assemblages relative to each other by one first controllable reactive chemistry (where control is exerted through the positioning of particular types of reactive groups in precursor structures and control over physical conditions or addition of reagents possibly including cross-linking agents), and possibly second and possibly third mutually distinct controllable reactive chemistries differing also from said fist controllable reactive chemistry, followed finally by formation of connections between any thus juxtaposed. (Up to three orienting connections may be desired to constrain sheets or three-dimensional assemblages into articulation of opposing faces, three being the number of points sufficient to define a plane.)

Alternatively, where multiple distinct affinity groups may be used in tandem, multiple orienting connections may be formed in the same step.

In the general case where a first (orienting) connection may be made in some manner controllably different from all subsequent connections, the construction of two- and three-dimensional structures may be accomplished by prepositioning all connecting chemical groups such that the distance between any two such groups, or at least between each of such groups and said first orienting connection, is unique. In this way, the two or three precursors interconnected in one hierarchial step may be correctly connected in a manner relying or spatial complementarity. Thus, after said first orienting connection is formed, only one relative angle at said connection between the involved precursors will permit the formation of

connections between any (and generally all) other connecting groups.

Further, all of the connections formed in the construction of two- and three-dimensional assemblages need not be of homogenous polarity, where such connections may be described as having a polarity (e.g. donor and acceptor reactive groups, where homogeneity would refer to all the reactive groups on one precursor being either donors or acceptors.) Such polarity may be another modality of complementarity of configuration of assemblages or precursors.

Where convenient separation of failure products or convenient monitoring of the purity of uniquely desired products is preferred, capping of unreacted connecting groups at the end of relevant hierarchial steps with detectable or purifiable reagents may be availed. This may be regarded as designed self-checking, error flagging or self-diagnostic capability.

These methods may thus be readily applied to the construction of asymmetric 2- and 3-dimensional particles or structures, which may have a great many uses including in polarizing optical devices such as liquid crystal displays.

These methods may further be readily applied to the construction of polymeric electronic devices from appropriate compounds. Polymeric electronic devices have been described,[123,124,125,126] but have heretofore relied on lithography or printing methods for fabrication and thus do not avail the advantages of polymer processability, nor the advantages which may be availed by the methods of the present invention. Through the use of appropriate protection-deprotection or activation polymerization chemistry and as desired affinity targeting, the present hierarchial methods may be readily applied to the fabrication of precise structures useful in any electronic application in which such compounds may be applied, including three dimensional array structures and three dimensional devices. Thus, rectifiers, diodes, transistors, gates, capacitors and other electronic components may be produced from appropriate monomers, oligomers or polymers. Devices comprising such components may be constructed (as further described herein) by the methods of the present invention, and may further comprise solid state components such

as nano- or micro-crystallites, colloids, or other particles, as further described herein.

Further, structures comprising superconducting crystallites or nanocrystallites, or superconducting organic compounds, including polymers thereof, charge-transfer salt complexes thereof, mono- or multi-layers thereof or polymers comprising said superconducting organic compounds as side groups or inclusions.

Further, where superconducting properties are thus conferred upon the molecular components or assemblages of the present invention, said components or assemblages may favorably further comprise conventional conducting paths within their structure such that when said components or assemblages do not satisfy conditions necessary for the superconduction regime (e.g. due to conditions such as temperatures above the critical temperature for superconduction, T_c, or intrinsically induced or external magnetic fields, devices comprising said molecular components or assemblages may fail gracefully or continue to operate without the benefit of superconduction (e.g. continued operation with lower performance).

Additionally, molecular electronic devices[127,128,129,130], whether they rely on single molecular segments or ensembles of molecular segments for electronic transformations (and including so-called molecular electronic devices the functioning of which involves proton or ion displacement or currents, photon conduction or interaction, charge density waves, spin-spin interactions or spin-density waves) may be constructed or fabricated by these hierarchial methods as well as other methods of the present invention. The methods of the present invention may further find use precisely in the development of such molecular electronic and related molecular computational devices, because these fields require methods and means to precisely arrange different molecular segments, moieties or complexes.

The methods of the present invention, favorably including the present hierarchial methods, may be applied to the construction of structures suitable for the implementation of the charge transfer devices described by R.C. Merkle in U.S. Patent Number

5,357,548 entitled "Reversible Charge Transfer and Logic Utilizing Them".

In addition, the methods of the present invention, favorably including the present hierarchial methods, may be applied to the construction of 1-, 2- and 3-dimensional devices comprising quantum dots and quantum anti-dots (which may, for example, be of solid-state, solid-state micro- or nano-fabricated, particulate, colloidal or polymeric composition), as well as quantum wires and quantum sheets, of appropriate composition. Thus, the methods of the present invention may favorably be used to construct many of the various quantum devices which have been proposed comprising such components, and may therefore be applied to the research and development of novel quantum electronic technologies, and the fabrication of devices designed through such efforts.

A. CONCURRENT EXPANSION OF ASSEMBLAGE STRUCTURE AND AFFINITY SPECIFICATION:

The present invention exploits targeted molecular recognition to accomplish the programmable assembly of addressable loci on molecular structures .

The present invention further exploits the hierarchial assembly together of components and assemblages produced according to the methods of this invention. Hierarchial assembly is advantageous not only because it renders the construction of highly complex heterogeneous structures synthetically tractable, but because it enables the use of modular building blocks (including both molecular components and assemblages thereof). This enables important economy of effort where general sets of components may be employed to produce objects of diverse design.

General sets of components will, however, by definition, be limited in the diversity of specificity of affinity groups they include. At minimum, said affinity groups must sufficiently distinguish all of the portions of such modular components to be separately addressable from each other. This may therefore pose problems of insufficient distinction of affinity groups of similar or identical modular components used recurrently in some particular assemblage.

This problem may be addressed in a number of ways which follow from the methods of the present invention. Both involve treatments of the affinity groups of such components (which may notably be hierarchially produced assemblages to further be built upon or utilized hierarchially) so as to further differentiate their specificity from otherwise identical components while retaining the desired degree of distinction of addressability of affinity groups of interest included within the structures of such modular components or assemblages.

The first and simpler method is essentially a special case of the use of bridging affinities, termed specificity expanding bridging affinities (SEBAs), where one portion of each SEBA is targeted to each first addressable affinity group of interest on some component or assemblage and one distinct portion of each of said SEBAs has a more differentiated addressable affinity which will be used as a cognate affinity group, having addressable correspondence to the respective first addressable affinity group, to which other molecular components or bridging affinities may in turn be targeted. Thus, two identical modular components may be addressably distinguished from each other while the information content of the structural configuration of all involved first addressable affinity groups is maintained and may be later used. Put differently, the addressability of particular regions of molecular components or assemblages may be maintained while specificity is further elaborated, refined, expanded or differentiated.

To illustrate this method, consider a "plus" shaped molecular component with a first uniquely addressable controlled affinity group at the terminus of each of the four arms of said component. For convenience, we may symbolize these as A,B,C and D, each complementary or having uniquely specific affinity to targeting affinity group segments A',B',C' and D', respectively. In this example, there are no other complementarities or affinity interactions. Targeting affinity group segments make up one portion of a SEBA, symbolized by the same letter represented instead in lower case. Each different SEBA contains a unique second segment which is a controlled affinity group of either distinct or greater recognitional complexity than those first affinity groups of the "plus"

component. Th se second segments may be symbolized as A",B",C" and D" respectively. Thus, SEBA a' will have a structure at least partly described as A'-A". One set of such SEBAs may be prepared and associated with an aliquot of said "plus" components. A second set of SEBAs with identical targeting affinity group segments but different, unique second segments may similarly be prepared. These second SEBAs may be symbolized identically to the first group but with the addition of a subscript "2" to each symbol. The second set of SEBAs may then be associated with a different aliquot of said "plus" components. The first uniquely addressable controlled affinity groups of each aliquot of the "plus" component may thus be said to have been addressably transformed to the addressable representation corresponding to the set of SEBAs used. Then a bridging affinity group having affinity to A" and A_2" may be mixed together with the two SEBA transformed (or associated or modified) aliquots of the "plus" components. This will form an association between one SEBA associated terminus (A-A'-A") of a molecule from the said first aliquot and an analogous SEBA associated terminus (A-A'-A_2"). If appropriate reactive groups had been incorporated in the initial "plus" component structure, these might be covalently linked together at such a stage of assembly by an appropriate cross-linking agent which will not modify any other portion of any molecule present in the mixture. With or without such cross-linking, the resulting structure will be a double "plus" assemblage with each terminus labeled by a distinct controlled affinity specificity, i.e. each arm will be uniquely labeled by one of B",C",D",B_2",C_2",D_2".

A second method of concurrent expansion of both assemblage structure and affinity specification or address differentiation may be accomplished by direct synthetic expansion of controlled affinity groups in components or assemblages. A subset or all controlled affinity groups within some assemblage are subjected to structural expansion comprising the addition of monomer or macromonomer units to one position (predetermined in the synthesis of the pre-existing controlled affinity groups' structures), or other specificity refining chemical modification.

This second method requires that all reactive groups and affinity interactions used prior to or subsequent to controlled affinity group expansion be stable to the reagents and conditions used to expand the sequence or specificity of those controlled affinity groups thus expanded. This condition may be satisfied by the use of protecting groups to prevent unwanted reactions at any susceptible chemical functional groups. Said protecting groups preferably either do not interfere with the desired binding interactions and molecular recognition to be effected by said controlled affinity groups, or will be readily removed to enable subsequent appropriate affinity interactions.

This second method may be most readily illustrated for controlled affinity groups of single stranded oligonucleotides as controlled affinity groups. For example, a "plus"-component such as the one above may have four unique and mutually non-complementary first controlled affinity groups which are each four base single stranded oligodeoxynucleotide moieties. Each of said oligodeoxynucleotide moieties may be attached, for example, to said "plus"-component through a single phosphoester linkage to one of the backbone phosphate groups otherwise involved in a phosphodiester linkage comprising the backbone of the respective said oligodeoxynucleotide moiety. Both the 5' and 3' ends of said oligodeoxynucleotide moieties are protected from oligodeoxynucleotide synthesis reagents, according to the appropriate oligodeoxynucleotide synthesis chemistries, which are well known to those skilled in the respective art. Said 5' protecting groups will be selected so as to be susceptible to removal by distinct treatments from those which remove said 3' protecting groups, but each of said protecting groups will be stable to the treatments which remove the group protecting the other terminus of the oligodeoxynucleotide moiety. Thus, by appropriate treatments, either all susceptible 5' or all susceptible 3' ends may be selectively activated for addition of monomers or macromonomers. Then appropriately protected monomers or macromonomers of the selected type will be added, and a phosphodiester linkage between these reagents and all of said oligodeoxynucleotide moieties permitted to occur, such that all deprotected termini of all oligodeoxynucleotide

moieties are identically modified by addition of the same nucleotide base or oligo- or poly-nucleotide. In a following step, the terminus of all such susceptible oligodeoxynucleotide moieties not modified in the foregoing step may similarly be selectively deprotected by appropriate chemical treatment, and the appropriate type of appropriately protected monomer or macromonomer may be added to effect a similar sequence expansion. Generally, expansion need only involve one terminus of such moieties. The discrimination of complementary affinity association for such oligodeoxynucleotide moieties, however, will be greatest for sequence differences towards the middle of such oligodeoxynucleotide moieties, and thus the greatest degree of unique addressability, which will be positively correlated with sequence length of said oligodeoxynucleotide moieties, will be achieved by locating the more local contributions to controlled affinity addressability towards the middle of such sequences, with the differences corresponding to the differentiation of targeting from one region of an assemblage to another located towards the periphery or termini of such sequences. The enhanced sensitivity of hybridization stringency to internal mismatches is a consequence of the cooperativity of interactions underlying such associations, and has been described by Sambrook et al.,.[131] On this basis, expansion of polynucleotide controlled affinity groups is most favorably accomplished as described, such that the expansion of said controlled affinity groups (which may also be described in terms of the information content of said controlled affinity groups' structures') and the hierarchiality of assembly may be readily mapped to each other.

X. CONTROLLED ATTACHMENT TO SOLID SURFACES:

Molecular components may include one or more functional groups that chemisorb or physisorb. For example, thiol groups (-SH) physisorb to various surfaces of metals including gold, and ions physisorb to charged surfaces. Generally, the greater the number of such association can be formed, the greater the cooperativity of binding and hence the strength of binding with respect to physical stresses and thermal dislocations. Thus, the sites at which an object produced by the methods of this

invention in relation to the remainder of its structure may be very well controlled.

In the case of highly extended structures which must attach with the surfaces of actuators, the actuators may be brought into physical contact with an approximately correct region of the extended structure, and scanned or vibrated so as to sample its local space; when contact is made, associations will form, cooperatively where there is more than one functional group in the region of said object to attach to said actuator. Thus a degree of self-organization or self-alignment may be availed in such attachments.

Such linkages to solid surfaces may be those utilized in solid phase methodological syntheses of the molecules in question, or ones formed to an intermediate or finished product not related to solid phase synthetic methodologies.

Affinity groups or molecules may also be deposited onto patterned surfaces as previously described.[132]

A particularly convenient method for the production of spatially patterned controlled affinity groups (which may thus be used to map molecular recognition to a microlithographically defined pattern) has been described by S.P.A. Fodor et al.,.[133] For purposes of the present invention and in general, the methods of S.P.A. Fodor et al., and analogous methods, may be improved by removal of a photoremovable protecting group[134] by spatially controlled exposure of a surface (and all molecules thereon) to a predetermined frequency and intensity of light by an instrumentation setup such as that used for Near Field Scanning Optical Microscopy (NFSOM) or similar near-field patterning techniques, which is capable of effecting patterned exposure with spatial control substantially better than one half the wavelength of the light frequency used, a limitation imposed by the resolution of far field optics.

Additionally, the method of S.P.A. Fodor et al. may be improved by the incorporation of photodegradabe or photocleavable linkages between the affinity groups synthesized by these methods and the surfaces to which they are attached such that removal of said affinity groups may be accomplished by illumination with appropriate frequencies which must be chosen to degrade said photodegradeable linkages. Said

photodegradeably linkages must be chosen so as to be unresponsive to illumination by any light frequencies used in deprotection during synthesis by these methods.

By use of such spatially controlled and patterned solid phase synthesis methodologies, or any functionally equivalent method, assemblages of the present invention may be controlled affinity targeted to complementary affinities thus distributed on a surface, with other modifications such as covalent bonding to prepositioned reactive groups (which may similarly be polymerized as a chemical group, protected or unprotected, on a monomer or macromonomer compatible with the chemistries of photolabile protected polymerization effected onto said surface) as provided by the present invention.

Thus, an assemblage with several moving parts, some providing mechanical power or motivation, others providing control and still others providing signal communication or data input or data output, may be specifically attached to different regions of a surface with control equal to or greater than that routinely achieved with microlithography. Said surface may be moving parts, and may particularly be parts of a Micro-Electro-Mechanical System. Patterns produced on such surfaces and those produced in assemblages (e.g. by hierarchial assembly with concurrent affinity expansion) may thus preferably be designed to match spatially.

A. PREFERRED EMBODIMENT: SCANNING PROBE MICROSCOPE TIP FABRICATION

A scanning probe microscope tip may be seen as a special case and a preferred embodiment, in which nanometer or subnanometer accuracy in positioning of a well defined molecular object is required. Thus, the methods of the present invention are capable of addressing both the construction or synthesis of such molecular objects and their immobilization and precise positioning onto the solid surface of a more conventional SPM probe.

Presently, scanning probe microscopy generally depends on a macroscopically produced or microfabricated probe or tip. Tips fabricated by macroscopic or microfabrication means are

generally not atomically sharp and generally do not have particularly regular surface configurations. This lack of sharpness and the lack of surface regularity convolutes structural information obtained from a sample with these imprecisions and random irregularities. It is therefore highly desirable that tips with high sharpness, regularity and reproducibility (of image quality thereby obtained) be conveniently fabricated. It is further desirable that the structure of such tips be well determined and/or well known, such that geometrical information may be used to enhance images thus obtained.

Many molecules display these characteristics of "sharpness", regularity and reproducibility of synthesis as well as being of well defined structure which may be determined by techniques well known within the chemical arts. Molecules thus present excellent candidates for use as scanning probe tips. Molecular components and assemblages of the present invention may be designed to meet these criteria particularly well, as well as to provide further advantages. The chief impediment to such use has been a lack of methods for localizing the binding of molecules to the apices of solid tips or probes. Suitable methods to accomplish such localized binding are described below.

Note, however, that the methods of the present invention may be used to produce structures of micron dimensions or larger, and thus produce structures comprising apices which may be bound to comparatively flat surfaces in the same way that pyramidal Si_3N_4 tips are sometimes deposited on otherwise flat cantilever surfaces. In these cases it will be understood that the molecular component member or members or structures thereof which ultimately comprise said apex and moieties located thereupon are, by the same token, targeted to said apex.

Note further that, while early embodiments refer to combination of molecular tips or arrays thereof with macrofabricated or microfabricated tips or probes, said molecular tips or probes may equivalently by positioned on other molecular components or assemblages of the present invention, or devices produced with the methods of the present invention.

i. Molecular tip and molecular tip array:

The methods of the present invention may be applied to a rigid, linear polymer, which may have properties for the conduction, confinement or channeling of quantum particles (e.g. electrons, photons) or states (e.g. spin density waves). Said rigid linear polymer may be the structural member of one or more molecular components to be used to build up a structure suitable for use as a scanning probe microscope tip or for hierarchial incorporation into a two dimensional array of such molecular tips. Note that such an array may contain a multiplicity of such tips, of different types as desired, with predetermined spatial localization into said array accomplished by hierarchial assembly. Any given molecular tip may comprise, preferably in proximity to its apical region, a particular affinity group, a particular biological receptor or ligand; in these cases, these functionalities may be used, with appropriate actuation and positioning means, to position molecular components with respect to each other or with respect to surfaces. Any given molecular tip may also comprise, again preferably in proximity to its apical region, a particular chemical group, a particular catalytic chemical functional group, or a particular enzyme; in these cases such tips may be used to modify molecular or supramolecular structures or assemblages, or surfaces.

Such a rigid linear molecule may be combined with a conformationally inflexible or reduced flexibility structural member comprising a controlled angle or vertex member. Such a vertex may be the structural member of a molecular component or employed as a cross-linking reagent. An example of such a vertex member is a phenylene ring with two polyparaphenylene molecules of controlled length and side chain distribution added to two adjacent carbon atoms of said rings, i.e. a member having a di-polyparaphenylene-o-phenylene backbone. Three such vertex members in molecular components may be combined, via targeting affinity groups of said molecular components or via step-wise macropolymerization and cyclization, to form a triangular molecular component. Either by stepwise macropolymerization or by affinity targeting, two such

triangular molecular components may be associated and joined, preferably via short linkers along one vertex of each of said triangular molecular components, permitting close connection between the two triangular molecular components but sufficient conformational freedom for bending of the plane of the first of such triangular molecular components relative to the plane of the second of such triangular molecular components. According to the distribution and mutual intercomplementarity of the incorporated affinity groups, two such bi-triangles may be bound together to form either a tetrahedron or a square pyramid. Note that this example would produce equilateral triangles (and in the latter case a square base.) By appropriate design but retaining tetrahedral or square pyramidal geometry, each edge of the resulting frame may be of a predeterminable length differing from other such edges; this permits the production of high aspect ration tip molecules.

Such tip molecules may be formed from components comprising additional chemical functional groups and/or affinity groups within their structure. For example, appropriate affinity groups may be included in appropriate molecular components used in the production of the above square pyramidal tip molecule such that each edge along the base of said square pyramidal tip molecule will have one or more unique affinity group. Addition of appropriate bridging affinity components or molecular components may then assemble such square pyramidal tips together in a hierarchial manner, possibly with concurrent expansion of affinity groups and incorporation of different tip molecule types, to form a two-dimensional molecular tip array.

Such arrays would be useful in scanning probe technology based data storage devices[135,136] or in scanning probe technology based fabrication and nano-fabrication.

A further class of tip molecules is related to dendrimers. In the same way that dendrimers may be built up from a starting monomer, a monomer with three or more protected (or mutually inert) polymerizable chemical groups may be added to one end of a first rigid linear polymer of controlled length. Either by deprotection methods or alternation of generation methods, additional rigid linear polymer molecules of controlled length

are added to the remaining free said polymerizable chemical groups of said starting monomer. This process is repeated to yield a highly branched structure which converges on said starting monomer and said first rigid linear polymer of controlled length. Said structure will resemble a tree, and may be termed an arborimer. After a number of repetitions of this process sufficient to yield arborimers of desired size, the growing surface is reacted with either some terminating molecule, which may be an affinity moiety or some monomer comprising a reactive chemical group; said terminating molecule is chosen to permit binding to the surface or modified surface of some microfabricated or macroscopic tip.

ii. Methods of localization of supramolecular or molecular tip and arrays thereof:

Two general methods fulfill the criterion of precise localization to a unique apex of an SPM tip. Both rely on the unique property of the apex of a macroscopic or microfabricated tip: its greater proximity to the substrate towards which it is advanced during ordinary scanning. Thus, the apex of the tip may be operationally distinguished.

In the first localization method, the tip molecule, produced by the methods of this invention, is positioned on a solid support. This may be accomplished by one of any number of methods. Three such prepositioning methods will be described.

The first method depends upon constructing said tip-molecule to have a chemisorptive or physisorptive functional group or collection of functional groups at the apex or proto-apex of the molecule. Such molecules are then accordingly associated with a substrate surface at low to moderate surface density, resulting in an apex-down orientation. The tip is then rastered during a slow approach towards the substrate, in a manner analogous to the very low force deflection mode of contact AFM described by Binnig et al.[137] Alternatively, tip molecules may be designed, by the methods of the invention, for example as described above, to associate together into a regular two dimensional array which may or may not be chemically or physically bound to a flat surface. Such a two

dimensional array will preferably have a minimum molecule-to-molecule spacing (center to center distance) gr ater than the width of the micro- or macro-tip apex. Thus, the two dimensional array establishes a distribution that ensures that exactly one tip-molecule will associate with the apical surface of the tip.

The second general localization method distinguishes the tip apex, and then attaches tip molecules from solution to the uniquely modified site. A number of modifications will be suitable. All of these depend on some sort of chemical change to the tip, either where it comes in closest contact the substrate or alternatively everywhere else. The physical and chemical attachment of the tip will have to be designed accordingly. A tip may be brought in contact with a flat substrate under very slight forces, and then reacted with some solution. The region of contact will not be accessible to the reactive molecules in solution. In this case, the reaction or association of solvent molecules must prevent the association of tip molecules, such that only the unreacted apex will be a site for attachment of tip molecules. This is therefore a surface deactivating treatment. While the tip remains in contact with the substrate, the solution is replaced with a sufficient wash and/or neutralization solution. Finally, the tip is exposed to a solution of tip-molecules, which may then attach correctly to only the apex. Cooperativity will tend to favor alignment. The area of the tip apex protected from modification will vary positively with the applied force of contact. A procedure activating the unmodified surface of the apex will not depart from this method. Similar localization methods may distinguish the apex by coating it uniformly and then scraping away the protecting coating by increasing the force of an AFM scan. Likewise, the apex may be marked by coating a conductive tip uniformly with an electron beam resist (positive or negative) and then modifying this resist by subjecting the apex to a tunneling (or field emission) current. In the case of positive resists, the resist layer may provide reactive groups for covalent immobilization of the tip-molecule. Similarly, electrochemical etching of a thin layer of a different metal to which the tip-molecule will not bind,

or electrodeposition of a thin layer of a metal to which the tip-molecule will uniquely attach, may serve to demarcate a sufficiently narrow apex region of a metallic tip. Note that many of the methods of this second category are physical modifications which have been proposed and demonstrated as nanofabrication methods to be performed by scanning probe microscopes, instead performed here inversely, by a homogeneous substrate on a tip apex which is to be altered or distinguished.

Note that for such apical modifications, colloids, which would generally have larger radii than those attainable for SPM tips by microfabrication or electron beam deposition techniques, provide a relatively smooth surface which may also have advantageous properties for tip-molecule attachment. Gold colloids of 10 to 25nm are readily available and in combination with various tip-molecule geomentries could still yield favorable aspect ratios, while immediately presenting a favorable surface to which a tip-molecule may chemisorb. Colloids are also advantageous because they are rather unlikely to pose multiple-tip problems. Thus, for example, a macroscopic or microfabricated tip or cantilever would be attached by adhesive means to said colloid, which would then be associated with a tip-molecule. Under some circumstances, such colloids may be particles used as the solid phase support in solid phase synthetic methodology whereby said tip-molecules are produced, i.e. the linkage to the solid colloid which will later be bound to a larger microfabricated or macroscopically fabricated tip is formed before the synthesis of the tip molecule is completed. More generally, modifications of tip-molecules may thus be made in situ.

A further modification of this procedure is particularly convenient and will yield well oriented and well defined tips. Microscopic spherical particles (MSPs) comprising highly uniform solid nanospheres such as those of diverse composition recently been described,[138] or dendrimers or other prior art microspheres may be used as an intermediate tip structure for positioning a molecular tip in the following way. Other particles deviating slightly or grossly from spherical geometry

may be used, but in these cases there is gen rally less information readily available about the precise spatial configuration of said particles. An MSP is derivatized by appropriate prior art methods with two affinity molecules, a first abundant affinity molecule and a second scarce affinity molecule, which may comprise further controlled affinity groups or reactive groups, to yield doubly labeled MSPs. Derivatization of said MSP must be performed such that attachment of said first abundant affinity molecules and said second scarce affinity molecules will be sufficiently strong to resist the forces applied in subsequent steps; prior art methods such as those of E.L. Florin et al., and G. Lee et al. provide examples of how this may be accomplished. Said first abundant affinity molecules are adsorbed to said MSPs at a concentration and stoichiometry such that each MSP will bind a large number of said first abundant affinity molecules; the precise number will vary according to the surface area of said MSP. Said second scarce affinity molecule is adsorbed onto the surface of said MSP at a concentration and stoichiometry such that most MSPs will experience attachment of either zero or one second scarce affinity molecules, and few if any MSPs will adsorb two or more second scarce affinity molecules. A first surface to which said second scarce affinity molecule binds either directly or which is derivatized with affinity groups targeted by said second scarce affinity group is exposed to said doubly labeled MSPs, and then washed. The molecules serving as said second scarce affinity molecule and (if any) as those modifying said first surface are chosen such that the interaction between them will be reversible; E.L. Florin et al. have shown biotin and avidin to have suitable reversibility for such purposes. All retained said doubly labeled MSPs are retained by virtue of binding by molecular recognition of said second scarce affinity molecules, and all MSPs lacking said second scarce affinity group are eliminated by washing. A macroscopic or microfabricated tip with a surface bound by either the affinity moiety of said first abundant affinity molecule or by reactive groups on the same said first abundant affinity molecule, or with a surface modified with some molecule which will experience sufficiently tight binding, with

some degree of specific molecular recognition, by said first abundant affinity molecule, is then used to gently and rapidly scan said first surface. This scanning serves to locate a doubly labeled MSP retained on said first surface, such that the apex of said macroscopic or microfabricated tip may be positioned directly above the retained said doubly labeled MSP. Said macroscopic or microfabricated tip is then advanced towards said retained doubly labeled said MSP such that said first abundant affinity molecules on said retained said doubly labeled MSP have the opportunity to bind to the surface of said macroscopic or microfabricated tip or targeted molecules deposited thereupon. After sufficient binding has been permitted to occur in this way, said macroscopic or microfabricated tip is withdrawn from said surface, and said doubly labeled MSP will be retained by said macroscopic or microfabricated tip due to the greater cooperativity and thus net strength of binding of said first abundant affinity molecules to the surface of said macroscopic or microfabricated tip compared to the strength of binding of said second scarce affinity molecule to said first surface; binding of said second scarce affinity group to said surface will thus be disrupted.

Note that said second scarce affinity molecule may comprise a molecular component of the present invention, and thus serve to localize a molecular tip or molecular tip array of the present invention by means of affinity interaction positioning optionally followed by chemical reaction and/or cross-linking. Said second scarce affinity group may comprise second moieties which may be individual molecules or complexes thereof, for example, biological or biological related molecules or macromolecules or macromolecular complexes, such as an immunoglobulin or fragment thereof, one or more enzymes or complexes thereof, a biological receptor or ligand or fragments thereof, or a small molecule which is the ligand for some affinity group; these cases are particularly pertinent to mapping the locations and analyzing the properties of biological structures including transmembrane receptors, which is of keen interest in the development of pharmaceutical compounds. Said second scarce affinity group may alternatively comprise a catalytic moiety (e.g. a chemical functional groups

appropriate to effect the catalysis of some reaction), a catalytic particle (e.g. a platinum colloid), an affinity complex with a molecular component or assemblage thereof comprising a reactive chemical group, or a functional group.

Note that tips comprising affinity groups may be used in variants of AFM of SFM to map a sample, which may or may not be a biological sample, for the distribution of affinity determinants. For example, a tip comprising an antibody or receptor ligand may map the locations of an affinity determinant such as a membrane bound moiety on the surface of a cell, and determine other characteristics of said affinity determinant such as lateral stabilization within the structure of said membrane, or sample characteristics such as elasticity of said membrane at the locus of said membrane bound protein.

For example, the above steps may be performed where said macroscopic or microfabricated tip may be coated with gold, said first abundant affinity molecule may comprise two free thiol groups (-SH) oriented relative to each other such that only one of said two free thiol groups may interact with the same surface, said MSP may be a gold colloid of submicron diameter, said second scarce affinity molecule may comprise one or more free thiol groups and a streptavidin conjugate, said first surface may be silicon nitride sparsely derivatized with biotin conjugated albumin. Alternatively, said first surface may be glass or mica with biotin labeled DNA oligomers or polynucleotides bound. This will yield a macroscopic or microfabricated tip with an apically bound gold colloid, which in turn bears an apically bound streptavidin molecule, with good downward orientation.

In general, where tip molecules are associated from solution, they may favorably comprise within their structure one or more rigidly oriented electrical dipoles which, under the action of an external electrical field, may align said electrical dipoles, and thus said tip molecules, in some orientation relative to the micro- or macro-tip.

The above methods will generally yield molecular tips with good downward orientation. Because, however, of the nanometer

scale irregularities in the surface of the macroscopically fabricated or microfabricated tips, the above methods alone will not provide precise downward orientation. Two distinct methods may provide such precision.

The first method relies on anisotropic etch methods of microfabrication. These methods selectively remove material from a crystalline solid along one preferred lattice axis, yielding surfaces that are nearly atomically flat. A pyramidal tip, such as is commonly microfabricated for use in contact-mode AFM, but with a convenient lattice plane perpendicular to the vertical axis of the tip, may be subjected to anisotropic etching near its apex, yielding a substantially flat apex which is generally smaller than one micron in width.

The second method relies on coating a tip produced by macroscopic or microfabrication means with a sufficient thickness of a ductile material. Once coated, such a tip is mounted on a vertical micropositioning apparatus opposing an atomically flat surface (e.g. freshly cleaved mica or freshly cleaved highly ordered pyrolytic graphite) towards which it is advanced. After said coated tip contacts said flat surface, the tip is advanced slightly further so as to apply pressure across the area of contact made with said flat surface. Where said ductile material is a metal, this may be done at an elevated temperature such that annealing occurs and thus the metallic lattice reconstructs in a manner consistent with a flat boundary thus produced. Such annealed formed coatings will generally have better stability than those formed without fully disrupting any bonds that correspond to the preexisting amorphous or differently oriented crystalline lattice of the coating material.

Tip molecules are then attached to the flat apical surfaces produced by either of these methods or any other suitable methods by the immobilization methods described above.

Note that care must be taken to ensure that the flattened apical surfaces are sufficiently small to ensure that, for tips on cantilevers, the tip molecule will be a unique apex for the range of angular deflections that the cantilever will experience under imaging conditions. This constraint must be observed to ensure that the multiple varying contacts are not

made between th tip and sample; the stringency of such a constraint will be related inversely to the vertical dimensions of the tip molecule.

Where molecular tip arrays such as those described above are to be employed such that only one unique tip is to generally interact with a sample at one time, the macro- or micro-tip surface may be that of a smooth sphere, produced, for example by the methods of K.K. Kim [139], which is secured to a larger macro- or micro-tip surface by adhesive or chemical methods, or may otherwise have three dimensional curvature. Said curvature will geometrically constrain one unique tip to be substantially closer to any substantially flat opposed surface. Said one unique tip will vary according to the precise angular orientation of said macro- or micro-tip relative to said substantially flat surface, such that adjustment of said angles (pitch, yaw) will geometrically select a particular tip for greatest proximity or interaction with the sample surface. Such an arrangement permits the positioning of an entire array of a conveniently large number of molecular tips relative to some nanoscale feature of a sample, with precise predetermined control over the exact tip which will interact with said sample, such that (a.) imaging or sample modification may be switched to a fresh molecular tip if a tip in use is damaged, without the need for time consuming large scale sample repositioning and search for the same feature; and, (b.) that multiple tips with different properties, at predetermined locations within the molecular tip array, may be brought into interaction with a nanoscale region of a sample, such that different tip-sample interactions may be examined, and such that different proximal-probe based chemical modifications of a sample by a specific, reaction controlling or effecting tip, of a well controlled portion of said sample, may be effected.

a. Cassette Tips and Positional Control of Molecular Components relative to workpieces.

Tips comprising substantially apical affinity groups such as the second scarce affinity groups above or any other approximately apical affinity groups, which bind directly or indirectly to other molecules (third moieties) which are not

initially part of a sample may be operated so as to change the said third moiety bound to said substantially apical affinity group. By such means, the portion of a molecular tip complex which interacts with a sample may be changed (e.g. by effecting affinity exchange reactions or by dissociating said third moieties from said substantially apical affinity groups and subsequently binding with different said third moieties, or by depositing said third moieties and reversibly or otherwise non-destructively severing communication of said third moieties with said substantially apical affinity groups.) In this case, said third moieties may be described as cassette tips. Said third moiety may alternatively comprise a reactive group or affinity group which, through proximity controlled interaction with a sample, may become bound to said sample at the thus predetermined location or region. Withdrawal of said tips from said sample may then disrupt the binding interaction between said second scarce affinity group and said second moieties. Such a method has been proposed by K.E. Drexler,[140] but methods and means for the positioning of appropriate species at the apices of scanning probes have heretofore been lacking. Examples of the disruption of affinity interactions involving molecules bound to AFM tips are found in the work of E.-L. Florin et al., and G. Lee et al. referenced herein.

A special case of particular interest occurs where said third moieties comprise a molecular component or assemblage of the present invention, which may be deposited on a sample surface or workpiece. A sample surface or workpiece comprising appropriate affinity groups, affinity determinants or reactive sites may thus be bound, by said third moieties comprising such a depositable molecular component or an assemblage thereof, at particular binding sites selected by means of positioning said third moieties by movements of the probe to which said third moieties are bound relative to said sample surface or workpiece. Said depositable molecular components or assemblages thereof are brought into sufficient proximity with said sample surface or workpiece to permit binding. After binding has had sufficient opportunity to occur, said probe is withdrawn. Where said depositable molecular component or assemblages thereof have bound to said sample or workpiece,

withdrawal of said probe will sever the binding association of said molecular component or assemblage thereof to said probe. Withdrawal forces may be monitored to detect successful deposition on said sample surface or workpiece and severance of said binding association of said depositably molecular component or assemblage thereof to said probe. By means of such proximal probe positioning, diverse structures may be produced from a limited ensemble of molecular components (which may be modular) comprising a limited number of distinct controlled affinity groups, with or without the further use of bridging affinity groups or cross-linking agents. Note that structures produced in this way are equivalent to other assemblages of the present invention for purposes defining the scope of this invention. Such assemblages may thus be combined hierarchially or otherwise used in accordance with the methods and applications of the present invention. Note further, that reactions between reactive groups may be controlled by the proximal probes used in this way, for example, by the use of photodeprotectable reactive groups and macro- or micro-tips comprising optical waveguides and controlled near-field illumination with appropriate frequencies of light controlled thereby. Note, however, that the rate at which such construction by proximal probe positioning may occur will be limited by the rate of proximal probe motions and the number of probes used (e.g. in parallel or processively, analogous to computational pipelining), and thus not in general be as efficient as the general methods of the present invention.

iii. Force Sensing Molecular Tips:

By the construction of specialized structures, molecular tips suitable for the direct measurement of tip-sample interaction force may be produced. This is accomplished by means of incorporation of appropriate functional groups, chemical moieties or assemblages of molecular components which may be conveniently monitored for some physical parameter and which are arranged within the structure of the molecular tip such that said physical parameter varies with conformational changes or stresses resulting from tip-sample interaction forces. Said appropriate functional groups, chemical moieties or assemblages

of molecular components used in this way may be termed sensing means or sensing components.

For example, consider the above square-pyramidal molecular tips with edges of linear rigid polymer composition and constructed so as to further comprise one modified non-base edge. Said modified non-base edge comprises a linear rigid polymer which extends beyond the apical ("top") vertex of said square-pyramidal molecular tips by some length. Thus, said square-pyramidal molecular tips constrain the movement of such a linear rigid polymer which extends beyond the apical vertex, such that it protrudes, to a first approximation, as a rod. This construction may be referred to as a pyramid constrained rigid-rod cantilever. The bending properties of said rod may be availed in the construction of a molecular cantilever. Of course, other such cantilever arrangements or equivalent compliance sensing arrangements are readily apparent. Note that cantilevers such as these may find favorable application in vibrating probe variations of scanning force microscopy.

a. Sensing by Coupling Interactions:

Said pyramid constrained rigid-rod cantilever may further comprise along the same edge comprising a first said linear rigid polymer which extends beyond the apical vertex of said square-pyramid a second linear rigid polymer which extends beyond the apical vertex of said square-pyramid but which does not extend as far beyond said apical vertex as said first said linear rigid polymer. At one position or at preferably regular intervals along the length of said first said linear rigid polymer, one or more first interaction sensing moieties are incorporated, covalently or by affinity interactions, at appropriate steps during molecular component synthesis, or by the construction methods of the present invention thereafter. One or more second interaction sensing moieties are similarly incorporated within the structure of said second linear rigid polymer, positioned for appropriate interaction with (e.g. generally in substantially direct opposition to) said first interaction sensing moieties.

For example said first interaction sensing moiety may be a fluorescent donor molecule or complex, and said second

interaction sensing moiety may be a fluorescent acceptor molecule, which interact, for example, by first order Forster coupling. Such interactions are generally held to comprise strong fluorescent coupling when said donor is less than 1.8nm distant from said acceptor and little if any fluorescent coupling of this type at larger separations. Such conformation sensing by observation of fluorescence-coupling is accomplished by exciting said donor during scanning and monitoring for photoemission from said acceptor. Greatest sensitivity for conformational changes will be achieved with structures comprising one or more donor-acceptor pairs at separations slightly smaller than 1.8nm. Thus, slight shifts in conformation that increase this separation distance will yield a non-linear decrease in coupling. Conformational sensing of this type is thus used with some homeostatic feedback mechanism such that tip-sample interaction force is adjusted to restore a set degree of coupling (i.e. a set proportion of coupling across all donor-acceptor pairs of a molecular tip, or number of pairs coupled); a particular molecular tip structure or design will therefore have a characteristic set of tip-sample interaction forces corresponding to the threshold for each of the one or more donor-acceptor pairs if used at one of these thresholds and if the threshold for each donor-acceptor pair is sufficiently separated from that of the corresponding nearest neighbors. Methods for providing said feedback are well known within the art of scanning probe microscopy. Alternatively, closely spaced thresholds may provide a continuous range of coupling within which a setpoint may be specified.

Alternatively, donor-acceptor pairs may be placed on an appropriate molecular tip structure such that tip-sample interaction forces increase coupling, and in this case optimal separation distances will be just slightly larger than the coupling threshold distance. Of course, other physical coupling phenomena may be used.

An alternative interaction comprises electron tunneling from a photoexcited donor, through multiple aligned hydrogen bonds joining two parallel rods (such as said first said linear rigid polymer and said second said linear rigid polymer, in analogy to the through-bond electron tunneling mechanism studied by

Beratan et al.[141] and by C.J. Murphy et al.,.[142]) The phenomena of electron tunneling with through covalent-bond transfer, through hydrogen-bond transfer and through-space transfer have been experimentally observed, measured and treated theoretically.[143]

Further, electron tunneling coupling comprising stacking interactions between delocalized molecular orbitals may also be used, particularly in cases where delocalized ring moieties attached to segments whose relative position change such that stacking is geometrically favored or destabilized. For example, instead of hydrogen bond donors and acceptors and coupling through hydrogen bonds, delocalized ring moieties may be spaced along two opposing segments such that as one bends away from the other, progressively more rings translate such that stacking is reduced due to the resulting unfavorable alignment. This may be visualized as two parallel combs with teeth in opposition and interdigitating with each other; as the angle of one comb is varied relative to the other comb, juxtaposed surface area of adjacent (opposing) teeth is progressively reduced. In the case of stacking mediated electron tunneling coupling, degree of coupling will be highly sensitive to such changes in angle.

Tunneling coupling may be monitored by one or more fluorescent donors on one member of the coupled first structure or structural region and one or more fluorescent acceptors on a distinct second structure or structural member arranged so as to couple (e.g. by hydrogen bonding or stacking) with said first structure or structural region. Similar arrangements have been used to study electron transfer coupling in double helical DNA.[144]

b. Sensing by Interlock Closure:

Changes in the position of a molecular segment cantilever may also be sensed with a rotaxane or interlock. As above, consider a square-pyramidal molecular tip comprising one longer rigid linear polymer edge extending beyond the apex of said pyramid and one shorter rigid linear polymer edge parallel and adjacent to said longer rigid linear polymer. These together comprise two rigid linear extensions of said edge beyond said

apex, as above. A rotaxane is in communication with one of the two rigid linear polymers extending beyond said apex via one or more macrocycle moieties such that the corresponding linear polymer guest moiety, which comprises both narrow and wide regions, both of which may translate through the interior said one or more macrocycle moieties, passes in between said two rigid linear polymers. Said linear polymer guest moiety is preferably oriented approximately perpendicularly to said two linear polymer extending beyond said apex. Thus, translation of said linear polymer guest moiety through said macrocycle moiety entails translation of said linear polymer guest moiety relative to said two rigid linear polymers extending beyond said apex. Said rotaxane and the corresponding said linear polymer guest moiety is positioned such that as the distance between said two rigid linear polymers extending beyond said apex is changed by tip-sample interaction forces, said translation of said linear guest moiety through between said two rigid linear polymers will be modulated, i.e. said translation will be impeded as said distance is reduced, or alternatively, said translation will be less impeded as said distance is increased. Reduction of said distance impeding translation of said linear polymer guest moiety through said one or more macrocycles is the same class of interactions exploited in molecular mechanical digital gate interlocks described herein. Thus, said modulation may readily be detected with molecular mechanical digital logic, which may thus transduce said modulation to digital data (e.g. by monitoring the frequency of translation of said linear polymer guest moiety.)

Alternatively, such rotaxane based tip-sample interaction force sensing means may provide for detection according to the fluorescent coupling of an appropriate fluorescent acceptor dye moiety to a fluorescent donor dye moiety, one of which is carried on the molecular structure of said linear polymer guest moiety and another of which is attached to a fixed point of the tip-molecule assemblage such as one of said macrocycles. For example, in such a case said linear guest moiety may translate due to thermal energy relative to said one or more macrocycles; modulations impeding such random translations will either

incr ase or decrease said fluorescent coupling as fluorescent donor and acceptor moieties experience a change in displacement probability distribution function, which will result, according to specific circumstances, in increases or decreases in the probability distribution of separation distances between said fluorescent donor and fluorescent acceptor moieties. Said increases or decreases may be detected by, for example, fluorimetric means, particularly, but not necessarily those which avail single photon detection.

iv. Extensible Molecular Tips:

By attaching a structure such as a linear polymer near the furthest extension of a molecular tip toward a sample, and application of a tension directed away from said sample to said structure, deformation of said molecular tip may be effected. Such a structure, especially if it comprises a linear polymer, may be controlled by the interlocks of the present invention. For example, said linear polymer may comprise several collinear widened regions and pass through several interlocks. Adjusting the tension and the state of said interlocks may effect digital control over the extension of the apex or any other extremity of a tip molecule toward a sample. Said extension may be used to implement Z-positioning (referring to the conventional terminology used in the field of scanning probe microscopy). Said tip molecules may further additionally or separately comprise elastiomeric or other compliance means within their structure, which may further be responsive to some chemical or physical parameter, which may be controlled externally. Said extension may implement a component of Z-positioning if other means of Z-positioning are additionally provided. Z-positioning of this type will be especially favorable when used to control the extension towards a sample of one or more molecular tips within a molecular tip array.

v. Additional applications:

Note that the molecular tips produced by the methods of the present embodiment and invention may be utilized in both conventional and also microfabricated scanning probe microscope devices, but also in devices based on analogous proximal probe

sensing and recording techniques utilized in mass data storage applications. An example of the latter is found in the recent invention of Swila, J.W. Jr.[145], disclosed in U.S. Patent Number 5,307,311, though there are numerous similar and analogous scanning probe based data recording and retrieval methods which have been suggested or implemented, which may similarly benefit from the present embodiment and the present invention.

Numerous lithographic techniques involving the use of scanning probes to expose or scrape a masking layer or resist layer have been proposed and/or implemented. These are sometimes termed scanning probe lithography or scanning probe nanolithography. A shortcoming of these methods is variation in probe geometry, which may be improved by the use of the present methods and colloids, molecular tips or molecular tip arrays in place of conventional microfabricated or macrofabricated tips. Further, in such applications, molecular tips or tip arrays may comprise catalytic moieties which degrade polymeric resists.

Note that such nanolithographic methods based on scanning probe lithography may be combined, favorably with methods for the light directed photodeprotection controlled synthesis of surface bound affinity groups such as those cited or described below, and the above methods for positioning and depositing of molecular components from substantially apical affinity groups. Such combinations may be further favorably effected using the same instrumentation.

B. PREFERRED EMBODIMENT: ATTACHMENT OF MOLECULAR COMPONENT PORTIONS OF ASSEMBLAGES TO ACTUATORS

Attachment of the component molecules and assemblages of the present invention to actuators (including microactuators or nanoactuators) may be effected so as to power, control and temporally and spatially coordinate movements and interactions of said molecules or assemblages.

In particular, said actuators may be components of integrated devices. Examples include Micro-Electro-Mechanical Systems such as those fabricated by J.J. Yao et al.[146] which have been

shown to permit oscillations in displacement with megahertz rates. Other types of capacitive, electromotive, electrostatic, quartz crystalline, piezoelectric, invar or integrated variations or combinations of these may similarly be used as actuators which may be specifically attached to components of the assemblages of the present invention to accomplish highly responsive external positional control over said assemblages or over said components relative to the other components of said assemblages. Such positional control may be used to communicate force, energy and power to said components or said assemblages, where said assemblages comprise mechanical machines, and may be used to control the mechanical, conformational or configurational states of said components or said assemblages. Thus positional control of this kind may be used to represent and communicate data to said assemblages or devices. Analogous mechanical powering and control schemes have been described for interfacing to nanoscale systems by Drexler.[147]

Note that a particularly convenient form of actuation for purposes of the present invention, and in particular for use in the mechanical digital systems and devices of the present invention for purposes of data input and control effectuation, comprises actuation of moving parts (molecules) of molecular or supramolecular components or assemblages thereof with external electrical fields. Here, a rotaxane or similar topological compound comprising one or more charged atoms (i.e. anionic or cationic chemical functional groups) is bound directly to the surface of a conductor, which may be part of a microfabricated device which preferably comprises a digitally controlled array of such conductive contacts. Said charged atoms are positioned within the structure of the member of said rotaxane not in direct communication with said surface of a conductor. Thus, the member of said rotaxane comprising said charged atoms (which are preferably of the same ionic polarity) may move relative to the member of said rotaxane directly bound to said surface of a conductor, but these motions are constrained by said member of said rotaxane directly bound to said surface. Application of alternating fields may thus exert forces on said

charged atoms and thus develop tensions and/or displacements in said member of said rotaxane not in direct communication with said surface. These effects will be favorably enhanced where said charged atoms are not in ionic association with counterions (which may be achieved in many ways known to those skilled in the chemical arts) or where the quantity of counterions present is reduced or minimized to achieve a desired modulation of effective charge, and especially where said charged atoms reside or may enter the double layer region of said surface, where fields are highly inhomogeneous and more intense than at further distances.

Note further that such electrical field control over molecular actuators comprising charged atoms may also be embodied by compliant components such as elastiomers, entropic springs or mechanopotentiators. Here, controlled compliance components may operate in either or both the tensile or compressive regimes according to the polarities and electrostatic interactions employed.

C. PREFERRED EMBODIMENT: INTEGRATED DEVICE PROCESS TECHNOLOGY—ATTACHMENT OF WIRE AND SCAFFOLDING TO MICROELECTRONIC AND ELECTRONIC COMPONENTS

Electronic components ranging from discretes to integrated microcircuits, and including wire and scaffolding made by procedures that include microlithography steps may be targeted to each other in controllably specific manners, associated together, self-aligned and firmly connected (including the formation of conducting connections, by methods subsumed under the general procedures of the current invention.

Where solid or large particulate components to be attached together or to some other assemblage are larger, the mobility of said components in solution will be proportionately reduced, reducing the number of collisions between such components per unit time. A simple solution to this problem is any of the agitation methods commonly used in chemical reactions. This will, however be limited by the ability of assemblages to resist shear or mechanical stress without suffering damage. Gentle fluid flow of one component past a less mobile second component (e.g. bound to a solid phase employed for synthetic

purposes or entrapped in a matrix that reduces its mobility compared to other components) may also be applied.

The above approaches, however, may not fully address the fact that where more precise associations between solid particles are desired, for example between the end of a wire structural member and a particular region of a flat surface that may represent only a very small fraction of the total surface area, most random collisions will be unproductive. This aspect of the problem may be addressed simply by allowing longer times for association reactions, especially if one simultaneously separates and collects the desired product as more reactants or component particles are added to the assembly mixture.

A more efficient, precise and well controlled approach, however, relies on self-aligning association processes mediated by extended controlled affinity groups (ECAGs). As with the general form of this invention, these controlled affinity groups are localized by one of the above listed methods, by any other appropriate method of known art, or through hierarchial assembly, to the location on said solid surface at which attachment of one or more components is desired. The attachment of these ECAGs to said larger components or solid surfaces also occurs through a controlled association resulting in attachment at a particular portion of these molecules or complexes. In this embodiment, however, which may clearly be applied to any large object or assemblage (solid or otherwise), the ECAGs will project out from the point of attachment to said surface, such that a relatively large volume is spanned or occupied by said ECAGs. Said volume occupied by ECAGs, which may span several cubic microns or more, is limited only by the shear properties of said extended controlled affinity groups under the manipulations and conditions to be used. These ECAGs will generally, but not exclusively, be extended linear or sparsely branched copolymers, which associate with other extended molecules in a manner that is determined by the composition or sequence of each of said molecules.

These ECAGs effectively perform three functions in this step: (1.) sampling a comparatively large volume for a complementary ECAG to which it is specifically targeted; (2.) forming a nucleating association with said targeted ECAG; and (3.)

processively propagating said association along the length of the associating ECAGs such that the surface regions to which each ECAG is bound will be drawn closer and finally collocalized as the association is completed. This third feature of this step, whereby self-alignment is effected, may be regarded as a process of specifically zippering together complementary ECAGs.

A concrete example will illustrate this step. A solid component, such as a microfabricated transistor with different chemical functions or affinity groups patterned lithographically onto controlled surface regions, with different chemical functions or affinities located on each domain or type of semiconducting material, such that the collector, emitter and base (or source, drain and gate) regions are differently labeled.

Let us consider the case where distinct oligonucleotides have been specifically patterned onto said surfaces. These are selected so as to uniquely hybridize with the distinct termini of three different, substantially long DNA molecules, each of which has no appreciable homology to either of the two other DNA molecules and thus will not hybridize with any region of either of the two different DNA molecules. The corresponding double stranded DNA molecules are annealed to the labeling immobilized oligonucleotides, such that the specificity of labeling is communicated through said distinct DNA molecules. Chemical or enzymatic treatment is then performed to form covalent linkages between the termini of said DNA molecules and either the respective oligonucleotides or the surface regions to which the respective oligonucleotides are attached. After said covalently bound (or otherwise thermostably attached) DNA molecules have been thus bound, a denaturation step is performed to remove the complementary DNA strands which are not covalently bound to said transistor. Said complementary strands may be said to be protecting the strands which are more securely attached to the respective target regions. (Alternatively, schemes involving specific protection of particular ends, some variants of which may notably be effected simply by the covalent attachment step above, in combination with treatments with particular single stranded exonuclease

enzymes, may be used to selectively d grade and thus remove said complementary, protecting DNA strands. Such alternative schemes may comprise specific equivalent steps which would be obvious to one skilled in the art of recombinant DNA techniques once any one such scheme, such as the one above, is described.) The unbound denatured strands are then removed by washing or eliminated by one of any number of manipulations obvious to one skilled in the relevant arts.

Components which have thus been labeled with one or more ECAGs targeted to different large components labeled with other ECAGs to which one or more of the former ECAGs are targeted are then mixed together or otherwise brought into physical proximity, and conditions which permit association of said ECAGs are effected. In the above example where DNA molecules are used as ECAGs, such association conditions are hybridization conditions chosen such that only fully complementary DNA strands associate.

After labeling the three terminals of a particulate, solid-state transistor by the methods of the above example, it may then be ECAG-bound to micro-wires, prepared by known methods and also specifically labeled at its termini and at any desired nodes, such that these components are positioned in the uniquely predetermined manner in a desired relative orientation (e.g. orientation directed by two or more distinct collocalizing affinities on the two structures targeted together such that only one orientation is possible) and connectivity. At this point, optional chemical cross-linking of the collocalized contact regions may be performed to strengthen the bonding together of said components beyond the attachment strength provided by said ECAGs may be performed; there are numerous methods for accomplishing this stronger attachment, which are obvious from related art and from other methods of the present invention.

Note that such connections should be sufficient to form tunneling junctions between conductive or semiconductive materials.

After such binding of ECAG targeted components occurs, for assemblages of components to be used in electronic applications, stronger electrical connections may be formed by

the polymerization of conductive polymers from seed or initiator monomers which had been prepositioned at the mutually targeted regions of said components, such that polymers originating on each component polymerize together forming a conductive connection. Alternatively, electroless plating methods such as those described in the related art may be used to form a good electrical connection; multilayer coatings such as those described in related art or the present invention may be used where chemical functionalization of the contact surfaces forms the first layer of such multilayer structures, effectively wrapping said multilayers around the contacts of interest, and where said multilayers include layers of coordinated metals; or, by the use of electrical contacts to some part of the circuit assemblage thus collocalized, *in situ* specific electroplating or electropolymerization[148] may form good electrical contact by "growing" or "healing" components together.

Note that any electrical or electronic components of appropriately small size may be assembled by the above methods. Thus, the above methods could be used to fabricate a dynamic random access memory (dRAM) bit cell from appropriately prepared wires, transistors and capacitors; said bit cells may then be assembled together hierarchially by appropriate permutation of ECAG specificities (e.g. ligating longer duplex DNA molecules onto shorter ECAGs or other oligonucleotides specifically positioned in the initial steps by lithographic techniques) such that address lines and bit lines are targeted together in, for example, a controlled head-to-tail manner which is repeated hierarchically and with protection to ensure that associations are stepwise and thus numerical control over length or extent may be maintained. One-, two- and three-dimensional arrays of said bit cells may then be specifically targeted, in register, to the surfaces of more conventional microfabricated, integrated electronic devices, and thereby interfaced to them. Or different assemblages serving as address decoders, multiplexers and sense amplifiers could be attached to said bit cell arrays to form dRAM devices exclusively by the methods of the current invention.

This embodiment therefore represents an integrated device implementation technology, which bears analogy to the various CMOS, NMOS, PMOS, ECL, CML, Josephson Junction process technologies or analogous technologies in the relevant miniaturization arts. Note that such prior technologies have been combined with Micro-Electro-Mechanical-System (MEMS) technology; the implementation technology of the present embodiment may similarly combine electronic and electromechanical components, or may be used to assemble purely mechanical components. Note also that the electronic components thus assembled may be solid state, polymeric[149,150,151,152] (amorphous or crystalline) or of a polymeric type prepared by the methods or the present invention. Integrated device designs previously implemented in prior art integrated device implementation process technologies my therefore be implemented in the integrated device implementation technology of the present embodiment in a manner that will be generally straightforward to those skilled in the arts of integrated electronic device technology who have learned the general procedures of the present invention.

Note that the methods elaborated within this embodiment similarly apply to any instance where large components with correspondingly low diffusion rates in solution and correspondingly many ways of colliding together are to be joined together in a well controlled, self-aligned and oriented manner within a convenient timescale and without extensive handling or intervention in the assembly process. These elaborations may, for example, be applied to large assemblages of molecules produced by the broader methods of the present invention.

While DNA is used as a specific example of an ECAG, it will be noted that many biological or modified biological molecules or other affinity molecules are equivalent compositions for this purpose, and that any molecule or system of molecules with similar or analogous binding nucleation and processive association properties may be used.

Where the length of ECAGs is favorably large and shearing becomes an important consideration, ECAGs may be constructed as follows: bundles of identical length linear polymers (for

example comprising polyethylene glycol segments) chosen to be of some convenient length and decorated at some position along said length by a number of one unique specificity of controlled affinity group (such as an oligonucleotide) may be successively coupled in an end-wise fashion to similar bundles of identical length polymers with a number of one different unique specificity of controlled affinity group. ECAGs may be built up in this way to be of desired length and have desired sequence of unique affinity groups which may be chosen to be complementary to the analogous sequence of similar ECAGs. Thus any shear sensitivity inherent to the phosphodiester bonds of naturally occurring DNA may be removed from the linear structure of ECAGs, which are further strengthened by being bundled without sacrificing affinity complementarity.

Note that bonding between solid members thus assembled may be effected by controlled electroplating "healing" or by the eutectic bonding methods described by A.-L. Tiensuu and S. Johansson[153] wherein two adjacent solids comprising a nearly eutectic mixture, favorably Au-Si, are thermally bonded together. This latter case will be especially favorable as a processing step in the construction of microelectromechanical systems (MEMS) by the methods of the present invention.

D. PREFERRED EMBODIMENT: LOCALIZATION OF FUNCTIONAL COMPONENTS NEAR SURFACES—INTERFACING WITH SENSORS

Devices of the present invention may comprise outputs which facilitate signal communication or microfabricated or macroscopic devices. Any conveniently monitored change in molecular or supramolecular conformation or configuration may suffice.

A particularly convenient method and means for effecting such output signaling comprises the incorporation of fluorescent dye donor and acceptor moieties on members or molecular components of assemblages which move relative to each other according to the signal state to be relayed. As an example we may consider a simple binary signal. Said fluorescent donor and acceptor moieties are positioned relative to each other on components

which move relative to each other such that a first fluorescent coupling is effected when said components are in a first position relative to each other and no fluorescent coupling or a distinct second fluorescent coupling is effected when said components are in a second position relative to each other. Said fluorescent coupling may be effected by signaling commonest comprising rotaxane-like structures which in turn comprise a fluorescent donor or acceptor moiety on a macrocyclic or tubular member and a fluorescent acceptor or donor moiety on a linear guest polymer molecule moiety; where signals larger in intensity than those obtained for single molecules are desired, multimers comprising either or both serial or parallel arrangements of said signaling components actuated by a single logic line.

For example, said single logic line may be in communication with a first linear polymer guest molecule such that tensions applied via said single logic line may cause sliding of said linear polymer guest molecule through said macrocyclic or tubular members of said signaling components, where said macrocyclic or tubular members are anchored to a fixed structure. Said linear polymer guest molecule may favorably similarly be in anchoring communication with said fixed structure but via an elastiomeric, mechanopotentiating component or other compliance effecting component which is positioned in between said fixed structure and the points along said linear polymer guest molecule where said macrocyclic or tubular members encircle said linear polymer guest molecule. Thus, when tension upon said linear polymer guest molecule is applied, according to the exact arrangement, translation of said polymer guest molecule may effect or disrupt coupling of said fluorescent donor and acceptor moieties. These changes in fluorescent coupling according to the displacements which the linear polymer guest molecules of such signaling components are predetermined to undergo in response to said tension, the distance or translation over which said tension is applied, the precise nature of the fluorescent coupling (which will be a property of the donor and acceptor moieties chosen, the light frequencies chosen and the physical and chemical environment in which said coupling occurs), the precise relative locations of

said fluorescent donor and acceptor moieties in said signaling components, and the structure of said signaling components. Said structure of said signaling components may be designed, for example, to self align said fluorescent donors with said fluorescent acceptors (e.g. impose constraints on relative rotations of said macrocyclic or tubular members relative to said linear polymer guest molecules) and bound the translations of said linear polymer guest molecules relative to said macrocyclic or tubular members, such that minimum and maximum separation distances between said fluorescent donor and acceptor moieties may be enforced. Said fluorescent donor and acceptor moieties may favorably be chosen to display first order fluorescent coupling of the Forster type, which may display a threshold sensitivity for separation distances at approximately a few nanometers.

Such signaling components availing modulation of fluorescent coupling phenomena may favorably be employed with one moving member coupled directly or indirectly to a surface of a CCD, SLM or frequency specific light filtering device, preferably but not necessarily in an affinity targeted manner. When used in this way, photoemission from such fluorescent moieties may be detected in the near field regime and, also due to proximity, a larger portion of the spherical emission probability space will impinge upon such optical detecting means. Fluorescent excitation may be accomplished by the illumination of said devices (comprising fluorescent signaling components and optical detection means) from a distinct source of appropriate wavelength to effect said excitation but to which said optical detection means is either unresponsive or may distinguish from the wavelength of photoemission. Alternatively, said optical detection means may be an array device comprising an optical detection means array interleaved with an illumination means array such as a laser diode array, light emitting diode array or spatial light modulator with an external or integrated light source. In this case, a single integrated device may monitor signaling components which are specifically located (by affinity targeting to affinity modified microstructures by means of the methods of the present

invention or other appropriate prior art methods) on the surfaces of predetermined elements of said optical arrays.

Note that many other arrangement will be equivalent. For example, said fluorescent acceptor may be located on the surface of said optical detection means, and a second signaling component may instead comprise a controlled compliant component (such as a mechanopotentiator) bound by the means of the present invention to said surface and in communication with said single logic line carrying the signal to be transformed as output information, to which said fluorescent donor is attached such that in one position it will be within coupling distance from said acceptor on said surface but in a second position, which relates a different logical state, will be located at a separation distance greater than the threshold distance at which coupling is first seen to occur for said fluorescent donor and acceptor moieties under the conditions used. Here again, self alignment is availed by means of said compliant component, which may be complex and implement a non-linear compliance function for the operating ranges of tension and temperature.

Alternatively, piezoresistance changes in ultrafine piezoresistive microfabricated structures may be monitored to detect tensions directly applied to said ultrafine piezoresistive microfabricated structures by one or more logic lines attached directly thereto by the solid surface targeting and immobilization methods of the present invention. Similar physical phenomena have been exploited in the fabrication of AFM cantilevers with intrinsic force detection capabilities. Silicon whisker structures may favorably comprise such ultrafine piezoresistive structures.

XI. PREFERRED EMBODIMENT: OPTICAL ARRAY DEVICES AND OPTICAL INFORMATION DEVICES

The methods of the present invention may readily yield highly uniform and precise structures with control to nanometer precision, which is more than sufficient for far field optical

devices. Thus, ultrahigh resolution optical array devices are well within the applicability of the present invention.

1. Display Devices:

Display devices including those used in computer display and video display applications typically comprise some form of picture element array. Common picture elements for so-called flat panel devices are light emitting diodes and liquid crystalline displays. Light emitting diodes which may emit a wide range of light frequencies have in recent years been produced from polymeric compounds, one example of which is provided by M. Berggren et al.,.[154] Another example of electroluminescent devices consisting of organic active material and capable of emitting white light has recently been reported by J. Kido et al.,.[155] Structures comprising such polymeric structures may be constructed by the methods of the present invention. Other light emitting devices comprise solid state semiconducting materials, which may be assembled into two dimensional arrays by the same methods applied to the fabrication of microelectronic devices above, or other methods of the present invention. With appropriate materials, comprising either or both polymeric compositions including dyes or solid-state compositions, laser diode arrays may similarly be constructed.

The methods of the present invention are particularly well suited to the construction of organic dye laser array devices because said organic dyes may be comprised by hierarchially assembled three-dimensional assemblages of the present invention produced by the methods of the present invention. Ordering comparable to that obtained for crystalline organic compounds may be achieved by such methods, but in a manner which need not depend as critically as conventional crystallization on the precise configuration of organic compounds or contacts between said organic compounds, and thus compounds which do not conveniently align for purposes of mode enhancement and lasing may be oriented in configuration more favorable to lasing phenomena. Further, since such orientation does not necessarily depend on close packing, structures may be

designed to facilitate thermal transfer, which can be a limiting factor for the active cycle duration or device life of solid-state lasing systems, for example by the incorporation of dissimilar substances or by effecting heat transfer by fluid flow through such a matrix.

Display devices comprising liquid crystal light modulation means may be similarly fabricated, by fabricating wire members, switching device members and optionally capacitor members into an array which is of similar electronic design to those conventionally produced for such applications, which is controlled by appropriate display controlling means. A film of liquid crystalline material is then deposited on or across said array, and then a transparent conductive film which may be constructed by methods of the present invention or fabricated by conventional methods is placed in opposition to said array. Numerous variations are known in the relevant arts, which will be amenable to fabrication by the construction methods of the present invention.

Note that said wire members or contacts thereof which contact said liquid crystalline material may be coated with copolymeric sheets of the present invention which may serve to orient said liquid crystalline material. Orientation of liquid crystalline phases by anisotropized boundaries is conventionally accomplished by poorly controlled and poorly reproducible methods, such as brushing a surface with lambswool. Polymeric coatings have been used for this purpose, but have not yet supplanted the problematic lambswool methods, in all likelihood due to poor control of polymeric coating processes with respect to anisotropy of the polymeric surface thus produced. By the assembly of extended structures by the methods of the present invention, well controlled surface orientation may readily be achieved, such that overlaid liquid crystalline phases may readily be oriented by self assembly phenomena.

2. Light Detecting Arrays:

Array devices comprising various materials susceptible to electrical effects produced by the incidence of photons of light have been used to construct array devices which are

useful for image capture and conversion to digital and video formats. Said array devices typically comprise charge coupled devices.

3. Light Modulating Arrays:

Light modulating arrays frequently comprise liquid crystalline based devices similar to those described above. Light modulating arrays, often termed spatial light modulators, have applicability in many areas in addition to display application. These include several proposed three-dimensional optical data storage technologies, where addressing of volume elements comprises spatially controlled illumination of an optically active material region. Such devices may similarly be constructed by the methods of the present invention.

4. Optical Information Processing and Communicating Devices

It has been noted by M. Rüetschi et al.,[156] who have used SFM to form scratches in polymeric surfaces to a similar end of orienting overlaid liquid crystalline phases, that well controlled orientation of liquid crystalline phases may be used to fabricate optical waveguides to channel photons. Thus, appropriate assemblages (including cylindrical structures) produced by the methods of the present invention may be combined with liquid crystalline inclusion phases to accomplish the same end. Such waveguides may be used as components in optical information communicating devices and optical information processing devices.

XII. PREFERRED EMBODIMENT: INTERLOCKS, MOLECULAR MECHANICAL DIGITAL LOGIC GATES AND DEVICES:

K.E. Drexler has shown that mechanical digital logic devices of nanometer dimensions may provide density and speed advantages over contemporary conventional digital microelectronics. This digital logic implementation has been termed rod logic. He has described such devices as they would be implemented with assembler fabricated diamondoid technology,

which is not at present possible. Mechanical digital logic devices may, however, be implemented by the methods of the present invention from synthetically accessible molecules and molecular components produced therefrom.

The basic computational element, a digital logic gate, may be implemented mechanically with a structure, termed an interlock, that confines two or more extended approximately linear molecules which vary in width along their length, together such that said molecules are in close proximity along a small portion of their length, preferably in an approximately perpendicular relative orientation. These approximately linear molecules may be rigid, or may be flexible molecules under tension (flexible lines in the tensi' regime.) The interlock consists in the intersection of two or more channels, each of which confines at least one of said linear molecules. A portion of each of said linear molecules traverses said intersection, by which the interlock structure is defined. The interlock is designed, and constructed generally from polymers of macrocyclic molecules, such that only a limited number of widened regions of said linear molecules may occupy the intersection at any given time. The interlock accommodates only a fixed molecular volume, such that presence of a sufficient number of widened portions of linear molecules prevents the wider portions of any further linear molecules associated with said interlock from transport through said intersection. The simplest interlock therefore consists of two perpendicularly intersecting tubes, with a linear molecular segment included in the interior of each tubule, and with each linear molecule having within its structure a steric extension which is capable of passage through the intersection region of the interlock when any analogous steric extension of the other linear molecule does not occupy said intersection region. Each steric or widened segment is of sufficient size to impede the passage of another analogous segment through the intersection of the interlock when the former steric or widened segment occupies the interlock. Thus, one molecule may control the translation of another molecule through said interlock, and the interlock may be in either an open or closed state with respect to the transport or displacement of a second linear molecule

according to the position of a first linear molecule (the position of which determines the occupancy of the intersection region of said interlock.)

Each such linear molecule may represent a logical state or numerical value according to its displacement from some reference alignment, which may favorably be defined by self alignment with, for example, some motion arrestors or further interlocks at some different location along said linear molecule. Each of these linear molecules may serve as a signal carrying structure, by virtue of shifts in position of one portion of such a molecule resulting, generally at the speed of sound, in propagation of such displacements, according to conventional mechanics, to other portions of said molecule. Said linear molecules may therefore be termed "logic lines" in close analogy with wires or signal lines in conventional electronic devices.

Such logic lines may include within their length elastic members, which will have greater compliance than the rest of said molecules. By such elastic means, a logic line may be subjected to an axially directed force or tension at one location, which may either result in the translation of the full length of said logic line through an interlock without significant extension of said elastic means, when said interlock is in an open state with respect to this logic line, or result in extension of said elastic means when translation of said logic line is blocked because said interlock is in a closed state with respect to said logic line. Thus, by incorporation of such elastic means, translation of the portion of said logic line on the side of the elastic means opposite of said interlock will invariably occur upon application of a tension to said portion, but translation of the remainder of said logic line will depend on the state of the interlock. The displacement of said remainder of said logic line will thus reflect the outcome of the application of tension to the line and passage or blockage of translation by said interlock.

Where multiple interlocks occur along the length of a first said logic line distal from the portion to which tension is applied, with appropriate widenings of the structure of said line positioned relative to the positions of said interlocks,

complex logical functions may be implemented. Each of such plural interlocks may have a perpendicular "input" line capable of opening or closing the involved interlock with respect to said first logic line, which may be regarded as an output line with respect to the logical device and operation thus effected. Said logical operation will be a logical OR of the state of closure of each of said plural interlocks through which said output line passes. Any of said plural interlocks can block translation of said output line if in the closed state.

The logical inversion of an input (to an interlock) may be accomplished according to whether translation relative to the relaxed state of said input line effects the opening or the closure of said interlock by widened segments of said input line. In the relaxed state, therefore, lines that are logically true at particular interlocks will be either open or closed, while those that are logically false will be either closed or open, respectively, according to the convention of logical polarity applied. Due to the mechanism of this digital logic technology, however, all OR operations will necessarily concern the closed state of any interlock located along the length of a particular output line. Any digital logic function may thus be implemented according to DeMorgan's theorem using the appropriate combination of inversions and OR-ings.

Note that the stopper groups of conventional rotaxane compounds demonstrate the principle necessary for self alignment of logic lines with respect to interlocks: stopper groups (i.e. sterically hindering groups which will not pass through the macrocycles comprising the respective interlock) may be incorporated along the structure of logic lines to prevent translation of said logic lines through said interlocks beyond a certain point. Such self-alignment, in combination with control over the relative linear position of widened regions of said logic lines relative to each other, to said interlocks and said stopper groups (i.e. through control of the comonomeric and/or segmental sequence of said logic lines by prior art methods or the methods of th present invention, where relevant sequence features include narrow regions, widened regions and stopper groups), will be particularly useful in ensuring the correct logical complementarity of a

particular interlock with respect to the corresponding input and output lines. Thus, according to the positioning of widened regions along the length of said logic lines relative to stopper groups or other means of linear ref rence pertinent to the operation of said devices, a logic line may communicate a signal which will effect opening or closure of said (non-disabled) interlocks when said logic line is translated such that said widened regions occupy or exit the intersection region of said interlock; complementarity is determined by relative position of said interlock to the widened regions of said logic line, which are in turn positioned in a manner predetermined during the synthesis of the corresponding linear polymer guest members.

The work of G. Li and L.B. McGown[157], investigating the occupation of the central channel of various cyclodextrin molecules by various numbers of *all-trans*-1,6-diphenyl-1,3,5-hexatriene (DPH) guest molecules and the consequent self assembly of these complexes into tubular inclusion complexes, demonstrates that the principle of steric hindrance is of critical importance in the formation of said complexes. Because the formation of said complexes necessarily involves transport of DPH molecules into the interior of adjacent cyclodextrin molecules, it may be concluded that steric interactions (which may be additionally modulated by other intermolecular forces) may be utilized to control the transport (translation or passage) of molecules through such confined regions or spaces. By extension, steric interactions may thus affect transport or translation of widened regions of linear molecules though such confined regions or spaces.

Note, however, that steric interactions are merely one convenient case of repulsive interactions. Attractive interactions may also be substituted, in which case closure of an interlock comprises the occupation of said interlock by an attractive group such that when an appropriate or complementary attractive group of a different logic line is translated to the intersection region of said interlock, attractive interactions occur which impede further translation of said appropriate or complementary attractive group of a different logic line through said intersection region of said interlock. Steric

hindrance interactions may be replaced with any interactions that vary according to relative position of logic lines. Drexler **1992a** does not explicitly treat such modifications of his proposed so-called rod-logic, which is in any case described as implemented in diamondoid materials rather than the molecular components of the present invention, which latter are more conveniently produced than any nanoscale solid-state moving parts, and may be produced by presently existing materials and equipment by the methods of the present invention. Said any interactions may derive from atoms within or side-groups upon said logic lines interacting with features of other logic lines passing though the same interlock. For example, two logic lines passing through the same interlock may each comprise positively charged atoms to effect repulsive interactions instead of or in addition to steric features. It is important to note that since different types of interactions may involve different forces and force gradients, and may further be distributed less locally than steric repulsions (i.e. collisions), that complex interactions changing as a function of translation are thus possible.

This principle may be applied in a variety of molecular structural arrangements, by utilizing the construction procedures of the present invention. A particularly convenient design relies on the perpendicular intersection of two tubular assemblages (produced, for instance, by joining four such tubular assemblages with appropriately shaped ends.) Self-assembly, possibly facilitated by the affinity colocalization methods of the present invention, is used to "thread" logic rods or tension lines (which may be understood in analogy to wires) through tubular molecules. Modulation of solvent composition or temperature so as to adversely modify the solubility of said rod or tension line molecules will favor their inclusion as guest molecules into the interiors of tubular (host) molecules. Some examples of such manipulations of guest-chemistry have been demonstrated by A. Harada[158], and by Li and McGown[159]

Alternatively, such structures may be formed by the self-assembly of plural such macrocyclic molecules around said rods or tension lines (by methods such as those of Stoddart et

al.[160]), followed by their reaction together, by appropriate reagents, to form approximately tubular structures (by methods such as those of Harada involving reagents, or by using cross-linking agents or bridging affinities); direct affinity binding may also be utilized provided the resulting structure is of sufficient mechanical strength and durability with respect to the desired mechanical functions it is to perform, and provided that sufficient tubularity results. This may most favorably be accomplished through the use of alternation of complementarity of association of macrocycles or tubule precursors composed of a predetermined number of such macrocycles produced by self assembly onto rods or lines of predetermined length. By alternation of association or by blocking and deblocking techniques, numerical control of length may be exerted over the formation of extended tubular hosts incorporating the desired rod or line guest.

In general, interlocks will be in direct communication, preferably through covalent bonds, or the topological constraints of rotaxanes or controlled compliance components, with molecular components serving a scaffolding function, or with solid surfaces, such that their positions are either fixed, or vary in a controlled way, with reference to such fixed structures. According to factors such as these, the state of enablement or disablement of one of such said interlocks, and the state of such interlocks (open or closed) may thus be controlled by the position of the member or members which are not thus fixed.

As described in the discussion of mobile interlocks, all interlocks may further implement digital latches when said interlocks comprise a linear guest polymer member with one or more narrow regions between plural widened regions which may occupy the intersection region of said interlock between which the widened region of another linear guest polymer member may pass at the intersection region of said interlock. Here, closure of said interlock by said another linear guest polymer constrains said linear guest polymer member to positions or ranges of translations with a single narrow region, juxtaposed to said widened region of another linear polymer, occupying said intersection region of said interlock, which may be termed

a latching interlock. Latching interlocks may be fixed, mobile, and/or dynamically or chemically programmable.

Note that while the interlocks described here for use in mechanical digital devices serve to constrain the relative position of surfaces of features of logic lines (e.g. narrowed and widened regions) such that steric interactions such as van der Waals repulsive contacts may be enforced according to the linear displacement of said logic lines, said interlocks may similarly align structures comprising substantially linear polymer guest members juxtaposingly such that other types of interactions may additionally or alternatively occur. The utility of availing other interactions will be made clear below in the description of rotaxane and interlock based copolymer sequencing devices.

A. Example assembly of interlocks:

A simple interlock, therefore, may be formed from rotaxanes. A simple case uses identical rotaxanes modified with distinct affinity groups appropriately targeted to each other. Consider starting materials of: a linear polymer molecule with a central widened region of 1nm length or more (for example, a polyethylene linear polymer serving as narrow segments and a linear fused ring system comprising five or more benzenoid or cyclohexyl rings serving as widened segments, and optionally additionally comprising a terminal protected reactive group at the free end) with one end terminated by a sufficiently large stopper group (which may comprise a controlled affinity group), preferably bound to said linear polymer near but not at the respective terminus of said linear polymer as a removable side group; and, two distinctly labeled tubular segments with suitable distinct controlled affinity groups appropriately targeting each other, with appropriately positioned reactive groups of appropriate type for the formation of bonds required below, and favorably with internal steric groups that orient or self-align the widened regions of said linear polymer molecule when said linear polymer molecule exists as a guest in said tubular segments. Such a linear polymer with one stopper end is chosen to be of a length suitable to two such tubular

segments, and said tubular segments are chosen to self assemble onto said linear polymer; said linear polymer is further chosen to be of a length which will not accommodate association with a third linear segment. Said linear polymer with one stopper d end is exposed to an equimolar mixture of said two distinctly labeled tubular segments, which are in excess. Where yields are of critical importance, additional affinity groups in communication with said linear polymer may direct the assembly of said two distinctly labeled tubular segments onto said linear polymer; otherwise, products with improperly oriented tubular groups will be eliminated in subsequent steps. Where assembly is random, the resulting products of assembly with two tubular moieties (bimacrocyclic rotaxanes) will be of four types, at least one of which will be suitable for construction of interlocks; depending on requirements imposed by design and synthetic methodology, two or as many as all four of said types may be acceptable. A second stopper is then reacted with a terminal side group of the free end of said bimacrocyclic rotaxane, capturing the two tubular segments onto the terminally stoppered linear polymer. Each type of tubule may carry a specific affinity label, and the population of products may be affinity purified for species having both of these labels. Where said labels are near one end of said tubules, affinity purification may be designed to retain only birotaxanes with said tubules arranged (in a head-to-head orientation) such that said labels are near to each other. This may be effected with affinity labels which are individually only weakly bound by an affinity modified matrix, and hence poorly retained, but by cooperativity will be well retained when in the appropriate orientation or position relative to each other. Such head-to-head birotaxanes may have tubular members which have, by use of appropriately structured tubular starting materials, two or more reactive groups of predetermined type at or near the opening facing the other tubular member, preferably arranged at opposite poles of the approximately circular or elliptical opening; said reactive groups will be chosen such that the tubules used to construct one such bi-rotaxane will not r act with each other. A first aliquot of such birotaxanes may be treated with an excess of an

appropriate cross-linking agent in sufficient concentration to singly populate (and thus not cross-link) all said reactive groups. An excess of first bridging affinity group is bound to said labels located near the "head" opening of said tubules. A second aliquot of said such birotaxanes is reacted with a compound which will effect reversible protection of said reactive groups and bound to a second bridging affinity group which targets said labels near the "head" opening of said tubule. Said first bridging affinity is chosen to target said second bridging affinity. After these steps, said first aliquot is mixed with said second aliquot at low concentration and conditions are adjusted to permit the association of said first bridging affinity group with said second bridging affinity group. Low concentrations are chosen as a means (among other possible means) of favoring bimolecular over higher order associations. After sufficient time has been allowed for association, extent of association may be assessed by affinity purification. Depending on the precise structures used, it may be possible for said birotaxanes to associate in a parallel rather than an orthogonal orientation; associations of this type will be readily purifiable by affinity techniques due to the fact that some affinity groups will remain unbound in such parallel complexes. We may thus consider fractions not retained by such affinity purification to have an orthogonal orientation, and consist in bi-(supra)-molecular orthogonal complexes of birotaxanes. Said fractions not retained may be examined by microscopic or scanning probe microscopic methods to confirm this. Said fractions not retained may then be treated so as to deprotect the protected reactive groups from said second aliquot. Where tubular starting materials were chosen to have appropriate geometrical distribution of said reactive groups at or near one opening, cross-links may thus be formed in said bi-(supra)-molecular orthogonal complexes of birotaxanes such that each tubule is cross-linked to both tubules of the birotaxane of which it is not part. Thus, the sum of the four tubules of this product will exist in a cruciform configuration, and the corresponding linear polymer guest molecules of each bi-rotaxane will be centrally juxtaposed.

It will be noted that the above example, while directly concerning only the simplest type of fixed interlock, may readily be generalized, by the appropriate additional modifications of starting materials and appropriate additional steps, to the construction of the dynamically programmable interlocks described herein, for example by the inclusion of additional affinity groups on said tubular members such that a third aliquot may be treated with appropriate bridging affinity groups to target corresponding affinity groups on the tubular members of a first or second aliquot, such that each tubular member of such a head-to-head birotaxane is bound to a similarly positioned tubular member of another head-to-head birotaxane, and such that the resulting product from the mixture, binding and coupling of all three aliquots at low concentration is a tri-(supra)-molecular complex of bi-rotaxanes with a central birotaxane included between and orthogonal to two external birotaxanes. In such a complex, the linear polymer member of said central birotaxane is centrally juxtaposed to both of the linear polymer members of the parallel external bi-rotaxanes. Additional structural members may be added to such a structure to effect closure of the upper and lower surfaces of the resulting central region, if desired; this may provide favorable additional constraint on the translation of the widened regions of said linear polymer molecules through said central region, but is not strictly necessary.

Note that affinity and reactive groups at the termini of said linear polymer molecules or said stoppers may, for example, be used to target these to appropriately derivatized termini of other interlocks, to compliance modifying members such as molecular springs or mechanopotentiators, to structural members of lattices, to actuators or to sensors. Hierarchial assembly with concurrent expansion of affinity specificity may also be applied to such assemblages.

1. Synthesis of a simplified interlock:

In the preparation of modified cyclodextrins for incorporation into stationary phases in chiral resolution applications, G. Yi et al. have produced an intermediate having

features particularly useful in the synthesis of simplified interlocks, which said intermediate these workers refer to[161] as compound **17**, $6^A,6^C$-[Di-*O*-*p*-(allylocy)phenyl]-$6^B,6^D,6^E,6^F,6^G$-penta-*O*-methylheptakis(2,3-di-*O*-methyl)-ß-cyclodextrin, which comprises two p-hydroquinone functions at opposite sides ($6^A,6^C$) of one face of the cyclodextrin, with terminal ethene functions. It will be recalled that Stoddart and coworkers have used polyethylene glycol macrocycles additionally comprising two or more p-hydroquinone monomers to form rotaxanes and concatenanes about polyethylene glycol linear polymers comprising paraquat stations along their length, wherein the electron rich p-hydroquinone monomers stack with the electron deficient tetraionic paraquat monomers, in a self assembling threading process. It will further be recalled that Li and McGown[162]have formed rotaxanes by the self assembly of ß-cyclodextrin about *all-trans*-1,6-diphenyl-1,3,5-hexatriene (DPH), which further argues that ß-cyclodextrin should complex electron rich guests, such as p-hydroquinone. The ethene functions of said compound **17** may be reacted, using the chemistries of G. Yi et al., (Scheme II) with a series of cross-linking polyethers such as $H(CH_3)_2Si-(OCH_2CH_2)_n-O-Si(CH_3)_2H$. This will create a loop extending from the narrow face of said ß-cyclodextrin. Then a linear guest polymer stoppered at one end and comprising an electron rich station such as a central p-hydroquinone unit is permitted to threadingly self assemble into said ß-cyclodextrin, with the free end subsequently subjected to a stoppering modification, yielding a ß-cyclodextrin rotaxane modified with a loop extending from the narrow face of said ß-cyclodextrin. This compound is then permitted to self assemble onto a polyethylene glycol linear second guest comprising a paraquat stations, according to the rotaxane self assembly methods of Stoddart and co-workers. This second guest is then subjected to a stoppering reaction. The resulting complex comprises two approximately orthogonal guests occuping a single intersection region, the target topology of a simle interlock. Said stoppers may comprise widenned regions which may pass into the resulting interlock structure before full steric hindrance with wider prostions of stoppering groups and the macrocycle

exteriors prevents further centrally directed translation. Said wider regions may, for example, comprise segments such as -O-CH_2CH_2-O-CH_2N-(CH_2CH_2-O-C_6H_4-O-CH_2CH_2-O-CH_2CH_2)n)2-N-CH_2-O-CH_2CH_2-O-Stopper. Such a branched-converging structure is chosen to relax requirments for allingment which would result from a planar (e.g. phenylene or napthalene based) said wider region. Based on the results of Li and McGown, one such said wider region should be accommodated by ß-cyclodextrin, and one such said wider region plus one straight chain polyether should be accommodated byshould also be accommodated along with a straight chain polyethylene glycol in an intersection region with sufficiently large loop structure. All of said stoppers may be chosen to comprise terminal reactive groups for subsequent decoration with affinity groups or further block addition polymerization reactions for incorporation of the resulting complex into working assemblages, and the chain comprising said loop may, as desired, be designed to further comprise a reactive group for direct or indirect linkage to scaffolds or surfaces.

B. Mobile and Signal Coupling Interlocks:

Note that interlocks may be anchored to particular locations in or on the assemblages of the present invention in various ways: in a first case, they may be anchored only by the constraints obtaining on the input and output lines passing through said interlocks, due to the attachments of said input and output lines to other molecular components or assemblages, surfaces, actuators, particles or components; in a second case, they may be in direct communication with other structures, such as lattices or matrices comprised of linear or branched polymers which are preferably rigid, preferably by means of either affinity binding or the formation of covalent bonds between one or more tubular or macrocyclic members of said interlocks with said other structures; or, in a third case they may be constrained to move along another single linear polymeric segment structure by virtue of attachment of one or more tubular or macrocyclic members of said interlocks to distinct tubular or distinct macrocyclic structures through which said single linear polymeric segment structure passes,

where said attachment to said distinct tubular or distinct macrocyclic structures may comprise an affinity binding interaction or a covalent bond. Interlocks anchored according to said first case may be described as, and termed, unconstrained interlocks. Interlocks anchored according to said third case may be described as, and termed, mobile interlocks. Unconstrained interlocks may be used in place of mobile interlocks where movements of said unconstrained interlocks in more than one dimension do not affect device function.

Mobile interlocks and unconstrained interlocks may be used to provide degrees of freedom while simultaneously effecting the coupling or decoupling of the associated input and output lines, which permits energetic advantages over the digital operations possible with only spatially fixed interlocks. Mobile interlocks and variants of the above unconstrained interlocks may be in communication with other structures via elastiomeric components, mechanopotentiators or other controlled compliance means, which will be particularly useful where said mobile interlocks or said variants of the above unconstrained interlocks serve to couple logic lines or logic signals.

Mobile interlocks are especially useful for mechanically coupling logic lines to each other in a digitally controlled manner. A mobile interlock may couple one input line passing through said mobile interlock to a distinct logic line not passing though the same interlock intersection but in communication with one or more macrocyclic or tubular members of said interlock. Said one input line passes through said mobile interlock in a direction with a substantial vector component in the direction of mobility of said mobile interlock. Closure of said mobile interlock, by one or more input lines preferably orthogonal to said one input line, which will be t rmed control inputs, will effect coupling of and communication of said mobile interlock with said one input line for all displacements of said one input line which effect collisions of (i.e. where van der Waals repulsive contact forces occur between) the widened region of said one input line with portions of the widened regions of said one or more input

lines which effect closure of said mobile interlock. Thus, the logical state of said one input line may controllably be transmitted to said distinct logic line by coupling, when such a component is in the closed or coupled state, and the logical state of said one input line may controllably be isolated from that of said distinct logic line when such a component is in the open or decoupled state. The terms coupling interlocks and mobile interlocks are thus equivalent. Closure of such mobile interlocks by said control inputs may therefore be considered gating of the logical state and transitions thereof of said one input onto said distinct logic line. By means of such controlled coupling, energy dissipation and associated tensions may be reduced, and thermodynamic reversibility of computational or information transforming operations may be approached or achieved. For a discussion of reversibility of computation and other associated energetic considerations, see Drexler, **1992a**.

A preferred case occurs where said one input comprises multiple widened regions, which the widened regions of one or more control lines may interpenetrate with when said widened regions of one or more control lines occupy the intersection of said mobile interlock and narrow regions between said multiple widened regions of said one input line. In this case under these conditions, said mobile interlock is locked with said one input, and the mobility of said mobile interlock is thus constrained to the region defined by said narrow region between said multiple widened regions of said one input. A favorable special instance of this preferred case occurs where said narrow region is only sufficiently large to admit interpenetration of said widened region of said one or more control lines between said widened regions of said one input. In this instance coupling will be highly constrained to one narrow segmental region of said one input line. Thus, for mobile interlocks and for interlocks in general, where the corresponding linear polymer guest segments passing through said mobile interlocks and said interlocks in general, the guest-int rlock complex may exist in a number of closed states at least equal to the number of narrow regions of said guest, due to closure of said interlock alternatively with each of

said narrow regions of said guest passing through the intersection region of said interlock. Thus, interlocks comprising linear guest molecules with one or more narrow regions between two or more widened regions may serve as digital latches of binary or higher order.

For logic line coupling by such a mobile interlock, said distinct logic line, which may, for example, serve as an input to a second interlock, is in communication, preferably by covalent attachment, with one or more macrocyclic or tubular members of said mobile interlock through which said one logic line does not pass through. Said one logic line and said distinct logic line are preferably oriented substantially in parallel, since coupling will permit tensions exerted on said one logic line to communicate with and thus affect the translation or logical state of said distinct logic line; other arrangements are, however, both possible and potentially useful. Coupling of said one input line is effected by closure of said mobile interlock, such that for some or all positions or ranges of positions of said one input line, said distinct logic line, or any component such as a functional component attached to said mobile interlock, will translate in direct response to translations of said one input line. Thus, said mobile interlocks may comprise positioning devices or positioning means. Said positioning means may thus be under the control of digital computation systems in a direct and fairly seamless manner. Note that other molecular components or assemblages of the present invention or functional components may be positioned on said mobile interlocks, such that said mobile interlock may translate said other molecular components or assemblages as controlled by said one or more control lines and said one input line.

Note that mobile interlocks or coupling interlocks may be simple (i.e. fixed or nonprogrammable) interlocks, may be dynamically programmable interlocks or may be chemically programmable interlocks, which latter two are described below. In these cases, programming determines whether coupling occurs between said one input line and said distinct logic line according to the state of the one or more said control lines by determining whether occupation of the intersection region of

said interlocks by widened portions of said control lines by determining whether the volume said intersection region will be sufficiently small to constrain or prevent occupation of said volume by widened regions of said one logic line during said occupation. In other words, the state of said control lines may enable or disable the ability of said one input line to be coupled to said distinct logic line according to their enablement by further logic lines which have narrow and widened regions which may pass through the intersection region of said mobile interlocks, which thus are programmable mobile interlocks. A further discussion of such programmability of interlocks is provided below.

Note that mobile interlocks may be applied to lines carrying either digital signals or the mechanical equivalent of analog signaling, unquantized or variable displacements and/or tensions.

C. Example hard-wired digital logic block:

The connectivity of such interlock-based logic gates may be determined, for example, at the level of synthetic construction, including by the methods of the present invention.

For example, consider the construction of a 2-in NOR circuit from interlocks. In this example, a line which is blocked for transit through an interlock (i.e. does not pass through a closed interlock) will be defined to be in a 0 state, and a line which has undergone transport of some point along its length through the center of an interlock will be defined to be in a 1 state. A 2-in NOR may be constructed from a 2-in OR followed by an INVERT. A 2-in NOR may thus be comprised of two interlocks each having a single input line and sharing a single first output line, arranged such that tension along either of said single input line, directed away from said two interlocks (perpendicular to said single first output line) will cause closure of the respective one of said two interlocks by either the first or the second bulky regions corresponding to the respective said single input line. Said two interlocks are thus arranged, relative to said bulky regions of the two said

single input lines, such that when said two single input lines are not subjected to tension, no portion of said bulky regions occupies the center of either of said two interlocks, and such that the application of an appropriately directed tension will result in transport of the respective one of said bulky groups into the center of the respective one of said two interlocks, effecting closure of said respective one of said two interlocks. Said two interlocks are thus default-open interlocks. Said closure will prevent the translation of said single first output line in a direction that will draw any bulky regions of said single first output line through the center of said respective interlock (which has been closed by the respective said single input line) when the corresponding tension is applied to said single first output line to test for the state of closure of all those interlocks for which said single first output line serves as an output. At another position along the length of said single first output line there is prepositioned a third bulky region which may traverse a third interlock, which serves as an INVERT gate, to which said first single output line serves as an input. Said third interlock is positioned with respect to said first single output line and said two interlocks such that when transit of said first single output line through either of said two interlocks is blocked by either of said single input lines, said third bulky region will occupy the center of said third interlock, preventing transit of the second single output line which serves as the output of said third interlock. Thus, said third interlock is a default-closed interlock. When instead neither of said two interlocks is in a closed state, said first single output line will be permitted to transit either partly or completely through said two interlocks. Said third interlock, and said third bulky region along said first single output line are positioned such that transit of said first single output line through said two interlocks will result in said third interlock entering an open state. Thus, when neither of said single input lin s are pulled, to effect closure of either of said two interlocks, and said two interlocks are thus both in open states, said first single output line will respond to an appropriately directed tension

by transport through said two interlocks, which will result in said third bulky region being transported out of the center of said third interlock, which is the condition for an open state of said third interlock. When said third interlock is in an open state, said third interlock will permit transport of said second single output line when an appropriately directed tension is applied to said second single output line. In sum, pulling either of said single input lines will result in the prevention of transit of said second single output, while pulling neither of said single input lines will yield a closure of said third interlock, preventing translation of the final output line (said second single output line) and effecting the desired NOR functionality. Thus, particular logic circuits may be constructed by topologically arranging interlocks and logic lines in an appropriate manner by the methods of the present invention. Such logic circuits whose truth tables are determined by the formation of structural connectivity may be termed hard-wired. Such hard-wired logic circuits may be combined together, according to the methods of the present invention, as modular blocks.

D. Dynamically Programmable Digital Gate Interlocks:

More complex interlocks may accomplish the same fundamental signal control function, wherein one input line may selectively enable or disable such an interlock with respect to closure sufficient to block the translation of an output line passing through said interlock, while another logic line may control the internal state of the interlock, and thus, when said interlock is enabled by said one input line, control the translation of the corresponding output line. Said one input line may be termed an enable line. Such a dynamically programmable digital gate interlock will be enabled when a widened region of said enable line occupies a correspondingly larger portion of the internal intersection volume of said interlock, such that both the widened region of said another logic line and the widened region of said output line may not also simultaneously occupy the internal intersection volume of said interlock; thus, said widened region of said enable line

constrains the intersection volume of said interlock such that said widened region of said another input may control whether said widened region of said output line may pass into or through said internal intersection volume of said interlock. Said dynamically programmable digital gate interlock will be disabled when only a narrow region of said enable line occupies said internal intersection volume of said interlock because a smaller portion of said internal intersection volume of said interlock is occupied by said narrow region of said enable line (relative to said larger portion occupied by said widened region of said enable line above) because said smaller portion occupied will not preclude the simultaneous passage or translation of widened regions of both said another input line and said output line through said internal intersection volume of said interlock. When only a narrow region of said enable line occupies said internal intersection volume of said interlock, further internal intersection volume occupied by said widened region of said another input line will be insufficient to block passage or translation of said widened region of said output line, such that said output line may experience transport or translation through said interlock; thus, when said interlock is in the disabled state, said another input cannot prevent transport or passage or translation of said output line through said interlock.

Said enable line may favorably be in communication with an output line or bit line of a memory cell interlock of a memory cell array such as described below, or may comprise a continuation of said bit line. Thus, the pattern of enablement of the interlocks of a dynamically programmable digital gate interlock array may be directly mapped, and thus programmed controlled, by the data pattern stored in said interlock memory array. Thus, a memory array programs said dynamically programmable digital gate interlock array, and thus the potentially complex digital "circuits" and functional blocks implemented therein and thereby.

Compliance potentiating members or controlled compliance components, such as elastiomers, molecular springs, entropic springs or mechanopotentiators, or structures comprising

multiples or combinations of these, in either serial or parallel or other arrangements, are used to impart compliance properties at particular points along the linear polymeric structure of logic lines. Said compliance properties are generally availed such that tension applied to one portion of a logic line on one side of an interlock will result in translation of said logic lines through said interlock if it is in the open state or disabled, and will result in expansion of or compliance of said compliance potentiating members on the same side of said interlock. Thus, whether said interlock admits translation of said logic line, a point distal to said interlock and on the opposite extremity of said compliance potentiating member will extend in the direction of an applied tension. If said interlock is open said extremity will extend towards an applied tension because of translation of the corresponding logic line through said interlock; if said interlock is closed, said extremity will extend due to the expansion of said compliance potentiating member under said tension. This facilitates the design of mechanical digital gates. Further, by incorporation of compliance potentiating members at both extremities of a logic line (i.e. all interlocks through which said logic line passes occur between said compliance potentiating members of said logic line), energetic balance and compliance balance is achieved for both the case where translation of said logic line is blocked and the case where translation of said logic line is not blocked relative to tensions applied to said logic line at points distal from said all interlocks through which said logic line passes with respect to said compliance potentiating members. In such cases, energetic complementarity of operation is availed, in analogy to CMOS microelectronic devices. By such measures, thermodynamic irreversibility of operations may be reduced.

Programmable arrays of dynamically controllable digital gate interlocks may be formed by hierarchial methods, which may favorably include concurrent expansion of assemblage structure and affinity specification. Note that as with programmable logic arrays, interlocks along one logic line output may

interact with said logic line inputs such that said interlocks, and the logic line inputs that correspond to said interlocks represent inputs to the array gate constituted by the translation of said logic line output. Thus, each orthogonal layer of logic lines may implement a layer of gates in an array architecture. In particular, a first output line from one such layer may serve, in such an array architecture, as an input line to a column of interlocks in the subsequent such layer. Distal portions of said first output line are subjected to oscillation by communication with oscillating actuators. If all of the interlocks in said one such layer served by said first output line are disabled or open, said first output line will translate through said one such layer and effect closure of any enabled interlocks in said column of interlocks in the subsequent such layer that it serves as an input logic line.

A system comprised of such devices will generally comprise actuation means arranged so as to exert multiple tensions or forces in two or three orthogonal directions, in a temporally coordinated manner. Such actuation may effect powering and clocking of logical operations. Actuation coupled to input lines to interlocks may similarly effect control over the logical state of such devices, and actuators may thus comprise device inputs.

Note that an array of such dynamically programmable digital gate interlocks may comprise a digital device closely analogous to conventional microelectronic programmable array logic. In particular, so-called semicustom, gate array, field-programmable gate array (FPGA), programmable logic array, and dynamically programmed architecture devices present a set of convenient architectural paradigms. Arrays of interlocks, with appropriate architectural "layering" of successive arrays and means of interconnection of said array layers, may comprise digital devices closely analogous to field programmable gate arrays or other programmable devices. Where dynamically controlled interlocks are used, a particular schematic or logic architecture is implemented with such a device by entering a binary pattern that activates or deactivates gates (dynamically programmable interlocks) within said arrays of interlocks, to

effect logical gates and specify interconnection paths between different gates, arrays and functional blocks of arrays. Such architectural issues and designs of various field programmable logic devices are well known within the arts of electronic engineering and computer science. Note also that due to the advantages of the methods of the present invention, such array devices may comprise interlocks, FPGAs and functional units comprised of interlock based gates arranged in three dimensions. Great advantages of density may thus be enjoyed in addition to thus gained from molecular scale, and parallelism exploiting such density may thus be enjoyed in the design and architecture of devices constructed by the methods of the present invention.

In FPGA applications or implementations with dynamically programmable interlock arrays, such an array may be overlayed with memory bit cells, the state of which determines the enablement or disablement of a one-to-one mapping of interlocks. Here, said FPGA device, which may be highly complex, may implement complex digital hardware functions, which additionally may be modified algorithmically, due to events, stimuli or changes of states or programmed functions occurring internally or externally. It will be noted that such a case is favorable for the implementation of robust cellular automata.

Alternatively, structures such as those used for dynamically programmable interlocks may be modified such that an irreversible chemical or conformation change occurs upon certain translations of said control lines to effect comparatively permanent enablement or disablement of said dynamically programmable interlocks, which would no longer be dynamically programmable. This is in analogy to programmable but unerasable programmable logic array devices of microelectronics. In a further variation, said chemical or conformational changes may be reversible, but only with energetic barriers. This is useful where it is desirable to program a device by loading a binary interlock enable or disable pattern to a memory array, load said pattern to the corresponding dynamically programmable interlocks via control lines, effect stabilization of said control lines in one state

or another such that the states of enablement of said interlocks are fixed, and decouple said memory array from said programmable interlock array. Thus, a memory array may be used to program a logic array into desired operational states, which are fixed (or "burned in") either irreversibly or reversibly, such that the memory array may then be freed to store data, which may be operated upon by said logic array.

E. Chemically programmable Digital Gate Interlocks:

Interlocks may be constructed such that one portion of the surface bounding the internal intersection volume is omitted, but may be formed by the addition of an appropriate affinity group or molecular component, targeted to the appropriate regions of the structure of said interlock, which may be affinity groups, to effect such intersection volume bounding. In this case, the internal intersection volume is sufficiently small when bounded by said affinity group or molecular component to constrain occupancy to one widened region of a logic line and one narrow region of another logic line, or to two narrow regions of two logic lines, i.e. to preclude the simultaneous presence of two widened regions of two logic lines within the bounded intersection volume of said chemically programmable interlock.

Note that where such chemically programmable interlocks are combined into arrays and programming is effected by the binding of affinity molecules or molecular components to affinity groups incorporated in the structure of said chemically programmable interlocks, said affinity groups incorporated in the structure of said chemically programmable interlocks may be concurrently expanded during hierarchial formation of said array, such that a particular affinity molecule or molecular component may target a particular interlock or subset of interlocks within said arrays, to effect a particular logic circuit or system, the architecture of which is specified by a mixture of affinity molecules or molecular components, which together target, and thus enable, predetermined interlocks within said array.

Chemical programming may also refer to the modification of dynamically controlled interlocks wherein some physical or chemical treatment causes some covalent bond or other stable association to form that chemically freezes the position of said one input (of the foregoing description of dynamically programmable digital gate interlocks) relative to the intersection volume of said interlock. In such an arrangement, the enablement or disablement of an interlock will not be modified by system operation but is instead predetermined at the level of device fabrication. Said treatment may, for example, include: exposure to appropriate chemical reagents; exposure to appropriate cross-linking agents; exposure to appropriate bridging affinity components; exposure to appropriately targeted molecular components comprising at least one affinity group and one reactive group; where said interlock or said one input comprises a photoreactive chemical group, exposure to appropriate wavelengths of light; or, where said interlock or said one input comprises a thermoactivatable chemical group, exposure to appropriate temperature. Such treatments, and the corresponding interlocks, may be chosen and designed, respectively, to alter the constraint posed by the structures surrounding the intersection region, to either effect or remove structural constraints which enforce steric blockage of said intersection region by widened regions of the respective polymeric guest species addressed by these treatments.

Note that an array of such chemically programmable digital gate interlocks may comprise a digital device closely analogous to conventional microelectronic programmable array logic.

F. Controlled Positioning Means:

The methods and assemblages of the present invention may be used to construct controllable molecular positioning means, and the assemblages may likewise comprise controllable positioning means within their structure. Such positioning means may be of a diversity corresponding to or exceeding that of micromechanical and conventional macroscopic positioning means. Thus, the structure, design, composition and functional properties of positioning means may vary widely according to

their complexity. Therefore, any positioning means constructed or synthesized by the methods of the present invention or comprising within their structure the molecular components or assemblages of the present invention are equivalents for purposes of the scope of the present invention.

A simple example will be provided, for illustration and not limitation, but it is noted that any of the molecular components and assemblages of the present invention may comprise positioning means. Said positioning means may be used in manners similarly to those in which molecular tips and molecular tip arrays may be used. Said positioning means may similarly comprise interaction constraining means such as those described below.

For example, a first interlock, having perpendicularly arranged tubular members not fixed to any fixed structure, may accommodate as guests two linear polymer guest members such as those used as logic lines, preferably of rigid composition. Each of said two linear polymer guest members passes through one set of tubular members comprising said first interlock, such that said two linear polymer guest members and said first interlock substantially reside within a single plane. Thus, motions of each of said two linear polymer guest members perpendicular to the linear direction of said each of said two linear polymer guest members will cause said first interlock to slide along the other of the said two linear polymer guest members. Said linear polymer guest members are preferably of rigid linear polymeric composition or under sufficient tension to ensure a substantially linear configuration. Both of said linear polymer guest members thus may determine the position of said first interlock within a two dimensional region, according to the position of said linear polymer guest members as they are translated obliquely in said plane. A simple embodiment attaches each of said two linear polymer guest members via their termini, in a fully extended manner, to two linear sliding members each comprising a linear polymer segment, preferably of rigid linear polymeric composition and comprising one or more widened regions at a distance from the point of attachment of said each of said two linear polymer guest members. Said linear sliding members pass through plural

interlocks, which may either impede translation of said sliding members through any of said plural interlocks which are in the closed state, or effect latching between any two widened regions occurring along the length of said linear sliding members. The position of said first interlock is thus determined by said position of each of the two sets of said mobile interlocks.

Note that members with any structure comprising two substantially circular or tubular domains which may constrain the location of a linear guest structural member passing through either of said two substantially circular or tubular domains may substitute for said first interlock in the above example, and may serve such constraining functions in positioning means in general. For positioning means of this general type, it is merely required that said two linear polymer guest members be constrained together by means which constrains them at points along their length which remain at a substantially fixed distance, defined by said means, regardless of the translation of said two linear polymer guest members. Such constraint preferably occurs near their point of closest approach of said linear polymer guest members in a manner which permits slidable coupling of said two linear polymer guest members to each other. Said constraining means may thus position some one or more affinity group, reactive group, molecular component, interlock, macrocycle, rotaxane or assemblage or other composition at a controlled locus within said plane, defined here as the X-Y plane. Note that an interlock in communication with said constraining means, through which a third linear polymer guest member passes, may be used to effect the Z-positioning of some one or more affinity group, reactive group, molecular component, interlock, macrocycle, rotaxane or assemblage or other composition at a controlled locus within a three-dimensional volume. Note further that two such one-, two- or three-dimensional positioning means may act upon molecular components with various structures attached to them to effect angular positioning of said various structures in addition to positioning in one-, two- or three- dimensions.

Interlocks impeding or latching said widened regions of said linear sliding members may be digitally sel cted in a manner directly analogous to the decoding of address enable lines in a memory array: one or two out of many are sel cted for closure which will localize any appropriately translated sliding member. Translation of such sliding members is thus effected by actuators or the appropriate molecular components (e.g. linear motors, etc.) of the present invention, and said translation is constrained by said interlocks impeding or latching said widened regions of said linear sliding members.

Thus, such positioning means may serve the positioning functions critical to various molecular assembly devices proposed by K.E. Drexler (**1992a**). Such a scheme relying on direct control over interlocks provides a facile method of positional specification with digital data, such that the digital systems using the assemblages of the present invention or others produced by the methods of the present invention may seamlessly control such positioning means. Thus, positioning means and computer control means may be combined in the same assemblage. Assembly devices of this type may be used, in a manner directly analogous to that described for positional control of molecular components (or other particles or molecules which may associate with components of the present invention) relative to workpieces by molecular tips and molecular tip arrays. Note further that a particular assemblage of the present invention may comprise one or several operational units comprising such controllable positioning means or their equivalents.

XIII. PREFERRED EMBODIMENT: MECHANICAL REPRESENTATION OF BINARY INFORMATION WITH ERASABILITY AND RANDOM ACCESSIBILITY:

Interlocks with input and output lines comprising plural widened and narrow regions which may occupy said interlocks may be used to implement digital memory devices. Generally, multiple interlocks will be r quir d to implement a 1-bit memory cell, though interlocks interacting multiple logic lines at or near the same intersection region may suffice. All of

the interlocks comprising said 1-bit memory cell will have one particular bit-line comprising a logic line comprising a linear polymer guest molecule, which comprises said plural widened and narrow regions which may occupy said interlocks as either an input line or an output line. One or more interlocks will serve addressing interlocks, which are interlocks serving as gates addressing said bit line. Said gates addressing said bit-line preferably implement latching functions on plural narrow regions of said bit line. Thus, each 1-bit memory cell bit-line in a 1-dimensional bit array will have at least one of said addressing interlocks at some point along the length of said bit-line. Each 1-bit memory cell bit-line in a 2-dimensional bit array will have at least two of said addressing interlocks at some point along the length of said bit line. Each 1-bit memory cell bit-line in a 3-dimensional bit array will have at least three of said addressing interlocks at some point along the length of said bit line. Addressing may be accomplished either by logical operations which activate a memory cell for some logical operation on a logic line of the memory array or by selecting a logic line of the memory array to test the state of a particular memory cell, or some combination of these possibilities. Each addressing interlock serves to specify a single memory bit cell from all cells along the axis defined by the input line serving said addressing interlock. Thus, said input line serving said addressing interlock is a line which may intersect with zero, one or two other such said input line to enable the 1-bit memory cell at the corresponding point of intersection. In such cases, activation of a memory cell is a logical AND-ing of at least two of the corresponding addressing inputs to said memory cell.

A particular three-dimensional device will now be described. In general, addressing may be unitary, or may be accomplished in different manners for read and write operations. The latter case will be described in further detail. One bit of data is stored by said 1-bit memory cell according to the position of said bit line relative to at least one interlock along its length. Write addressing of said memory cell is favorably accomplished by use of latching interlocks comprising inputs in the X and Y directions. In this case, said bit-line extends in

the Z direction. When either or both of said inputs in said X and Y directions effect closure of said latching interlocks on said bit-line, the logical state of said bit line will be preserved.

When both of said inputs in said X and Y directions are translated so as to effect the open state of said latching interlocks, latching constraint will be removed from said bit-line and it will be free to translate under other influences. One terminus of said bit-line is preferably in communication with a controlled compliance member attached which is in turn to some fixed point in a manner appropriate to effect compliance between said terminus and said fixed point. In this instance, removal of latching constraint on the position of said bit-line will subject the state of said bit line to said controlled compliance member, which will either set or reset said bit-line and hence the data content of said 1-bit memory cell according to the complementarity of data representation used. At times during which said latching constraint does not obtain, said bit-line may be further subjected, from a second terminus or point distal from said one terminus, to tension determined by the tension upon and position of a logic line extending in the Z-direction (termed a Z-data line). Thus, the state of said Z-data line will determine the displacement of said bit-line and hence the logical state of the memory cell of which said bit-line is part, which will be captured when a latching constraint is again applied to said bit-line, for example by either or both of said inputs in said X and Y directions. Communication of said Z-data line with said bit-line may be permitted in such designs to become slack where said Z-data line does not extend (or tension) said compliance member. In this case, said Z-data line may be said to not set said compliance member.

Two major variations are possible at this point, which may either omit said compliance member or may avoid any slack state of said Z-data line. In a first variation, said bit-line may pass through one or more mobile interlocks which are in direct communication with said Z-data line and which may be coupled to said bit-line by closure by one or more logic lines, referred to as a Z-selects, which are located in the X-Y plane. Where a

compliance member is omitted from the design of said memory cell, and said bit-line is either sufficiently conformationally rigid (in a statistical sense) or is of rigid polymeric composition for a sufficient proportion of its length, said mobile interlock may be swept to an extreme position of its range of travel after removal of said latching constraint such that the potentially indeterminate displacement of said bit-line which is under no compliance constraint will not pose any problems of indeterminacy: sweeping of the closed mobile interlock may thus "capture" widened regions of said bit-line to the state of the thus coupled said Z-data line. The direction, and hence the resulting end-point of said sweeping may, among other methods, determine the value thus written to said bit-line. Readout of a memory cell occurs when said Z-enable couples said Z-data line to said bit-line. The displacement or translation of said Z-data line will thus be determined by the positional or displacement state of the bit-line to which it is coupled, and interlocks elsewhere along the length or said Z-data line may sample the resulting state of said Z-data line and thus operate one the datum thus signaled which is uniquely determined by the logical state of the uniquely Z-addressed said bit-line. In such a memory array architecture, readout of memory cells may occur in a plane-wise manner, in which case read-out may be highly parallel, or Z-data lines may be uniquely selected by opening X-addressing interlocks and Y-addressing interlocks along the length of each Z-data line to be enabled for the data readout operation. Thus, the state of said bit-line is tested (read) or modified (written to) through enablement of communication of said bit-line with said Z-data line and translation of said Z-data line, which for read operations will be impeded at a location that is affected by the translation state of said bit line, and which for write operations, where said bit-line is not latched in a fixed position or state of ranges of translations, will translate said bit line to positions determined by the translation state of said Z-data line, after which latching is performed to lock said data line in a fixed state. In such an example, said Z-data line is preferably parallel to said bit

line, or has components of translation in common with said bit-line.

In a second variation, not relying on the use of mobile interlock components, data logic lines passing through interlocks positioned such that the relative position of a widened region of said bit-line may be probed (for reads) or be constrained in translation (for writes) by a data logic line passing through a fixed interlock. Said data logic lines may intersect a two dimensional memory array plane, in which case the device topology is similar to that described above, or may be restricted to an array plane of said memory array devices. This latter case will be favorable for two dimensional array devices, which it is worth noting, are of favorable bit density compared to conventional dynamic RAM devices of current and anticipated generations despite the planarity restriction.

Note that other arrangements such as massive shift registers may also serve as memory devices. The architectures described herein are chosen merely for illustration rather than limitation. There are a very large number of variations of memory architecture, as well as architectures of other digital functions and devices, known within the relevant electronic and computational arts, which may be readily implemented in the molecular mechanical digital technology described herein and/or by the methods of the present invention.

The address lines referred to above are decoded address enables, rather than logic lines carrying numerical information specifying an array address. Address decoding may be accomplished at the level of actuator selection, in which case it may be accomplished by microfabricated electronic devices, decoded by the appropriate decoder functions capable of decoding binary information or other representations to individual units, which are selected logic lines in this case, such decoders as are known within the respective computer design and electronic arts. Where addresses are input to a molecular assemblage as encod d numerical information, said decoders may comprise interlocks, implemented in any of the ways described above, i.e. interconnection by molecular construction methods, chemical or physical programmability

("hard-wiring") or irreversibly or reversibly conformationally stabilization of control lines of control-line programmable interlocks. Data output to sensors, comprising, for example, optical array devices such as CCDs, may be accomplished with, for example fluorescence coupling moieties on data lines which couple or uncouple according to the state of translation of said data lines. Data and address input may be accomplished with actuators, whether microelectromechanical, rotaxane based, piezoelectric, or elastiomeric, or by other appropriate means.

XIV. ROTAXANE AND INTERLOCK BASED COPOLYMER SEQUENCING DEVICES, MOLECULAR SCANNERS AND INTERACTORS:

As indicated above in the description of the interlock components of the present invention, interlocks, and other components comprising macrocycles encircling copolymers or other structures which may or may not have substantially linear segments, may be used to juxtapose different molecular or particle surfaces such that two or more regions of said surface may interact in any way which is affected by such juxtapositional proximity or colocalization, which proximity or colocalization and thus the corresponding interactions may thus be constrained by the position, composition, structure and spatial location of said macrocycle relative to or along the structure of said copolymers or other structures, which may or may not have substantially linear segments encircled by said macrocyle, and physical or chemical condition under which such juxtapositions are performed.

The example of polynucleotide sequence determination according to such methods and using such means will illustrate this principle, but it will be noted that these methods and means may be applied generally, including application to interactions where one member of said interactions is a non-biological linear polymer, which may be organic or inorganic, or non-polymeric in composition. Note further, that while macrocycles are of primary interest among suitable means for such methods and means, any suitable structural means of encircling said copolymers or other structures will suffice. Thus, noose-like arrangements or adjustable loops may comprise

means for juxtaposition or localization where adjustability of encirclement is required for stable juxtaposition and corresponding interaction. Note that several such adjustable encirling means may be used in tandem to dynamically secure said structures, which may or may not have substantially linear segments, for juxtaposition and/or constrained interaction. Said encircling means are preferably placed under parametric or algorithmic control, preferably effected by means of interlocks at appropriate positions along the length of linear segments which said encircling means comprise within their structure, to ensure adequate securement of said structures but not damage, deform or become entangled with or caught by features of said structures. It will be noted that unlike macroscopic solids, at the molecular level catching by features is less likely because friction such as that occurring in macroscopic systems does not occur in a sufficiently localized manner except at very high contact pressures. Said encircling means, whether of fixed or variable circumference, serve to constrain said structures or said copolymers with respect to some other molecular surface, which may be the surface of the interior region of said macrocycle, or some portion of the surface of a distinct substantially linear segment passing through the intersection of said encirling with some other macrocyclic or tubular member in communication with said encircling member in a similar way to that in which the preferably tubular members of interlocks are arranged. In this latter instance, the structure is analogous to an interlock with a variable circumference macrocyclic or tubular member. Said distinct substantially linear segment passing through said intersection may comprise copolymers or other distinct surface features, including reactive groups, which may be interacted with said sample. Thus, diverse surfaces of diverse chemical moieties may be aligned with and scanned along a sample, and interactions between said moieties and samples observed or performed.

Note further that said interactions may comprise chemical reactions.

Note that where not inappropriate in this section, the terms macrocyle and macrocyclic will further comprehend tubules and tubular structures.

In a preferred implementation, said encircling means comprise a multistrand inclusion rotaxane having two polymeric segments passing through the center of a macrocycle, or a concatenane additionally comprising a macrocycle positioned along one of the cyclized polymers comprising said concatenane in a similar manner as in the case of psuedorotaxanes. Adjustment of the circumference of encircling means is accomplished by translating one or two of the linear polymer segments passing through the macrocyclic member comprised within the structure of said encircling means relative to said macrocyclic member. Said translation is analogous to drawing a loop through a knot which encircles two thread or rope segments of said loop. As will be apparent to anyone familiar with the topologies of knots and strings, many topologies will be equivalent; topologies amenable to convenient synthesis, e.g. comprising self-assembling interactions or prepositioning by chemical or affinity means, etc., are preferred. Encircling means implemented in this way may comprise within the strands capable of inclusion regions which are more stably complexed by said macrocycle; these will be preferred positions of said encircling means. Note further that a particularly favorable arrangement comprises two encircling means oriented opposite each other such that members encircled by both of said two encircling means may be shifted in position by increasing the circumference of the closed loop of one of said two encircling means while decreasing the circumference of the enclosed loop the other of said two encircling means. In this way, the loops of both of said encircling means may be maintained under tension, including during expansions and reductions of loop circumference of either of said two encircling means.

A. Preferred embodiments: Polynucleotide Sequence determination with macrocyclic means

Said sliding comprises movement of said macrocycle or said interlock relative to the fixed termini of said extended single stranded polynucleotide sample molecule, the ends of which are attached to fixed structures such that said polynucleotide is fully extended and linear (i.e. stretching destabilizes stacking interactions of bases, and single helical conformation

no longer exists due to stretching; attachment preferably occurring by means which admit axial rotation of the attached polynucleotide.)

A polynucleotide sample molecule is prepared by assembling a macrocycle or an interlock, which may have affinity means complementary to some segment of said polynucleotide or a polynucleotide or other polymer segment ligated to said polynucleotide sample at a predetermined terminus by means obvious to those skilled in the arts of DNA recombination or organic chemistry, by self-assembling threading of said polynucleotide sample molecule through said macrocycle or interlock (where said macrocycle or interlock around said polynucleotide has been designed to facilitate said self-assembly by appropriate structure and surface complementarity.) Said polynucleotide sample is converted by appropriate means, such as denaturation or exonucleolytic degradation (preferably following immobilization) to single stranded form. More than one of said macrocycle or said interlock may encircle a single said sample molecule, in which case interactions may be performed and monitored in parallel. After assembly of said macrocycle or interlock around said polynucleotide sample molecule or extensions thereof, the termini of said polynucleotide sample molecule are bound to two distinct surfaces which may be moved relative to each other with sub-micron precision. Said two distinct surfaces may be MEMS cantilevers, or one may be an AFM-like cantilever and the second a substrate surface.

Said macrocycle may comprise rigid members and be substantially elliptical or rectangular or otherwise extended in one direction and scanned back and forth in a direction preferably perpendicular to said polynucleotide sample. In this case, plural base pairing moieties are located within the interior of said macrocycle along said one direction in some predetermine arrangements, and the force or energy of scanning displacement is monitored. Increases or decreases in this force as a function of displacement are recorded, and these data are combined with knowledge of said predetermined arrangement of said base pairing moieties along the long axis

of said macrocycle. Alternatively, an interlock-type structure may be used to juxtapose a linear polymer guest member perpendicularly to said polynucleotide sample molecule at the intersection of said interlock such that polynucleotide nucleobases or equivalent nucleotide pairing moieties are opposed to the bases of the sample polynucleotide molecule, such that pairing favorably occurs when the bases in the intersection region of said interlock are of appropriate complementarity. Note that the presence, polarity or absence of charge along said linear polymer guest member may be altered (at the level of design, or by physical or chemical changes such as pH) to vary the strength of binding. Translation of said linear polymer guest member perpendicular to said single polynucleotide molecule sample will occur with energies and forces determined by base pairing, which may be observed, either directly or by allowing said linear polymer guest member to come to equilibrium with the base of said single polynucleotide molecule sample occupying the intersection region of said interlock, which translation may be monitored by means of coupling such as that used with molecular tips or via digital interlocks sensing the translation of widened regions of said linear polymer guest member upon which said nucleotides or equivalent nucleotide pairing moieties are positioned.

Alternatively, encircling means comprising a single base-pairing specificity in proper orientation for pairing with the bases of said appropriately immobilized polynucleotide sample may be slid along said polynucleotide sample, and the tension or energy of said sliding may be monitored and recorded as a function of the linear position of said encircling means along the length of said polynucleotide sample. Three or four such encircling means comprising a single-base pairing specificity may thus completely determine the base sequence of said polynucleotide. In this case, said three or four such encircling means comprising a single-base pairing specificity may be parts of an assemblage which controls their relative position and thus the pitch (i.e. number of nucleotides between the interaction region of each of said encircling means) in a predetermined manner such that information thus obtained about interactions with said sample by each of said encircling means

may be conveniently aligned by use of information about said predetermined manner in which said base-pairing specificities have been positioned on said encircling means.

The macrocycles or tubules, or the polynucleotide hosting macrocyclic or tubular members of the interlocks of the present embodiment may preferably be designed to display energetically favored interaction with the anionic regions (phosphates) of the backbone of said polynucleotide sample molecule such that translation of said sample through said macrocycle or said interlock occurs with some energy barrier which will enforce stepping of said translation such that said sample molecule may be aligned relative to said macrocycle or said interlock with angstrom tolerance. The sample sliding or translation process will thus require some energy which is preferably larger than thermal energy (but not so large as to break covalent bonds). Said sliding comprises movement of said macrocycle or said interlock relative to the fixed termini of said extended single stranded polynucleotide sample molecule. Thus, said macrocyclic moieties or macrocyclic or tubular members of the interlocks of the present embodiment are in communication with some reference point, which may be a surface of an actuator, which moves relative to the points at which said polynucleotide sample molecule are immobilized. Forces of stepping may be detected as forces between these two references in cases where it is desirable to monitor the stepping process.

Note that in both of these cases, said encircling means may be understood to comprise sample aligning scanning probing means. Thus, problems of immobilization of sample molecules for scanning probe microscopy and/or modification associated with many molecular samples may be circumvented.

Note that the macrocycles based on modified cyclodextrins such as the cyclodextrins displaying molecular recognition of specific features of nucleotides described by A.V. Eliseev and H.J. Schneider[163] show some properties favorable for use in such macrocycle or tubule based scanning interaction polynucleotide sequence determination methods and means.

XV. PREFERRED EMBODIMENT: MATRICES AND SCAFFOLDS FOR MATERIALS FORMATION AND NANOSCALE PRECISION STRUCTURING

The ability to form precisely structured two and three dimensional matrices or other two or three dimensional scaffold structures permits the well controlled spatial positioning of chemical functional groups, affinity groups or other particles or compositions, including nanocrystallites and microcrystallites. Said chemical functional groups, affinity groups or other particles or compositions may be used to direct materials formation processes such as crystallization and solidification, and thus comprise materials nucleation components and materials nucleation means.

As noted above, affinity groups may be obtained which have affinity to particular surface features of solids, such as particular faces of crystals. Said affinity groups include small diverse copolymers such as those which may be derived from epitope libraries or antibodies, though non-biological affinity groups may also have similar properties. Thus, said affinity groups positioned on a scaffolding may selectively bind and thus position particles with surfaces to which they have binding affinity.

Many biological proteins of keen biotechnological and biological interest elude crystallization, and hence the structural analysis possible with X-ray diffraction methods. Scaffolds produced by the methods of the present invention may position affinity groups, including antibodies or fragments thereof, and further, constrain rotation of said affinity groups. For example, a regular 3-dimensional matrix may comprise an immunoglobulin heavy chain member fixed at both ends along a lattice edge. An immunoglobulin species specific to a protein of interest are produced in L_1H_1 form by, for example, *in vitro* translation or other suitable means, as oppos d to the biological $(L_1H_1)_2$ valency. These are then permitted to react with said immunoglobulin heavy chain member fixed at both ends along a lattice edge. Thus, orientation along said edge may be constrain d incorporation of the appropriate generic affinity member in an appropriately

constrained orientation. Said protein of interest is then diffused into said scaffold and thus permitted to associate with said immunoglobulin species, thus precisely localizing its position, according to the structure of said scaffolding. Where said structure of said scaffolding is substantially periodic, the spatial positioning of said protein of interest will likewise be periodic. Note that said scaffold may be completely assembled before the introduction of said protein of interest, and further before said immunoglobulin species, or alternatively both of these or said immunoglobulin species may be permitted to associate with substantially two dimensional scaffold array components, which are then assembled, by the hierarchial methods of the present invention, into three dimensional structures.

X-ray analysis is then performed by comparing the diffraction of the scaffold with said immunoglobulin species present but said protein of interest absent with the diffraction of the scaffold with said immunoglobulin species and said protein of interest present and further bound to each other. In such a way, artifacts arising from unit-cell to unit-cell contacts in protein crystals prepared by conventional protein crystallization methods may be eliminated, and proteins which elude crystallization subjected to diffraction analysis.

Note that in the same way, not necessarily availing oriented binding of proteins by immunoglobulin species fragments, enzymes may be arranged within a scaffolding, which may further comprise means (e.g. alkyl or alkyl-vinylene chains such as are used to solubilize rigid polymers in organic solvents) for solubilization of said scaffolding in non-aqueous solutions, to form enzymatic microreactor inclusions. Similarly, reactors which constrain the transport of reactants to a channel, and comprise an arrangement of enzymes along the length or transport coordinate of said channel corresponding to the order of steps which are desired to be catalyzed to effect some overall multistep multienzyme reaction, may similarly be constructed by such aspects of the present invention; these may be referred to as multienzyme channeling assemblages (MCAs). MCAs are expected to improve overall reaction efficiencies and

rates by increasing effective concentrations of substrates, by protecting any unstable free intermediates and by more efficiently utilizing enzyme molecules by positioning them such that they occur only where needed (thus more sparingly). Such channeling has been observed in various natural multienzyme systems.

Affinity groups, including antibodies,[164] having specificity to particular solid surfaces have also been shown to nucleate the solidification of the correspondingly oriented surface, and where different solid phases exist having different solid surfaces, bias the formation of one solid phase relative to another compared to solidificatio.. not availing facilitated nucleation with affinity groups. Note further that nanocrystallites may serve the same function where favorable lattice matches between some surface of said nanocrystallite and a desired solid occur.

These phenomena may be exploited by the scaffolding construction methods according to the methods of the present invention to yield many useful possibilities for the production of advanced materials. First, said scaffolds may have useful mechanical properties such that the solids nucleated to form around and within them have advanced composite properties. Secondly, very well controlled microheterogeneity may be effected by control over the location and type of nucleations which occur. Thus, for example, a solid which exists in three different forms, each of which may be nucleated to form from solution by a particular affinity group or nanocrystallite or polymer surface, may be caused to solidify by such affinity groups or nanocrystallites with an arrangement spatially controlled by the positioning of each of said affinity groups or nanccrystallites. Such well controlled microheterogeneity may be expected to yield highly interesting and useful properties, such as, for example, controlled pinning in solid state semiconducting or superconducting materials.

Thus the methods of the present invention may be applied to materials fabrication, permitting vastly enhanced control and hence greater power to effect rational materials design.

The foregoing descriptions of embodiments reveals the general nature of the present invention so that others skilled in the appropriate arts can, by applying current knowledge, readily adapt or modify these procedures and means to any of a vast number of applications and with any of a large number of possible implementations without departing from the essence of the present invention. Such adaptations and modifications are therefore comprehended within the meaning and range of equivalents of the disclosed processes, means and embodiments. The embodiments and examples disclosed herein are therefore provided for purposes of description and not limitation. It is further understood that the terminology employed herein is similarly chosen for purposes of description and not limitation.

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CLAIMS:

As this invention may be embodied in several forms without departing from the essential spirit thereof, the breadth of invention is intended to be defined by the appended claims as opposed to the examples presented in the foregoing description,